

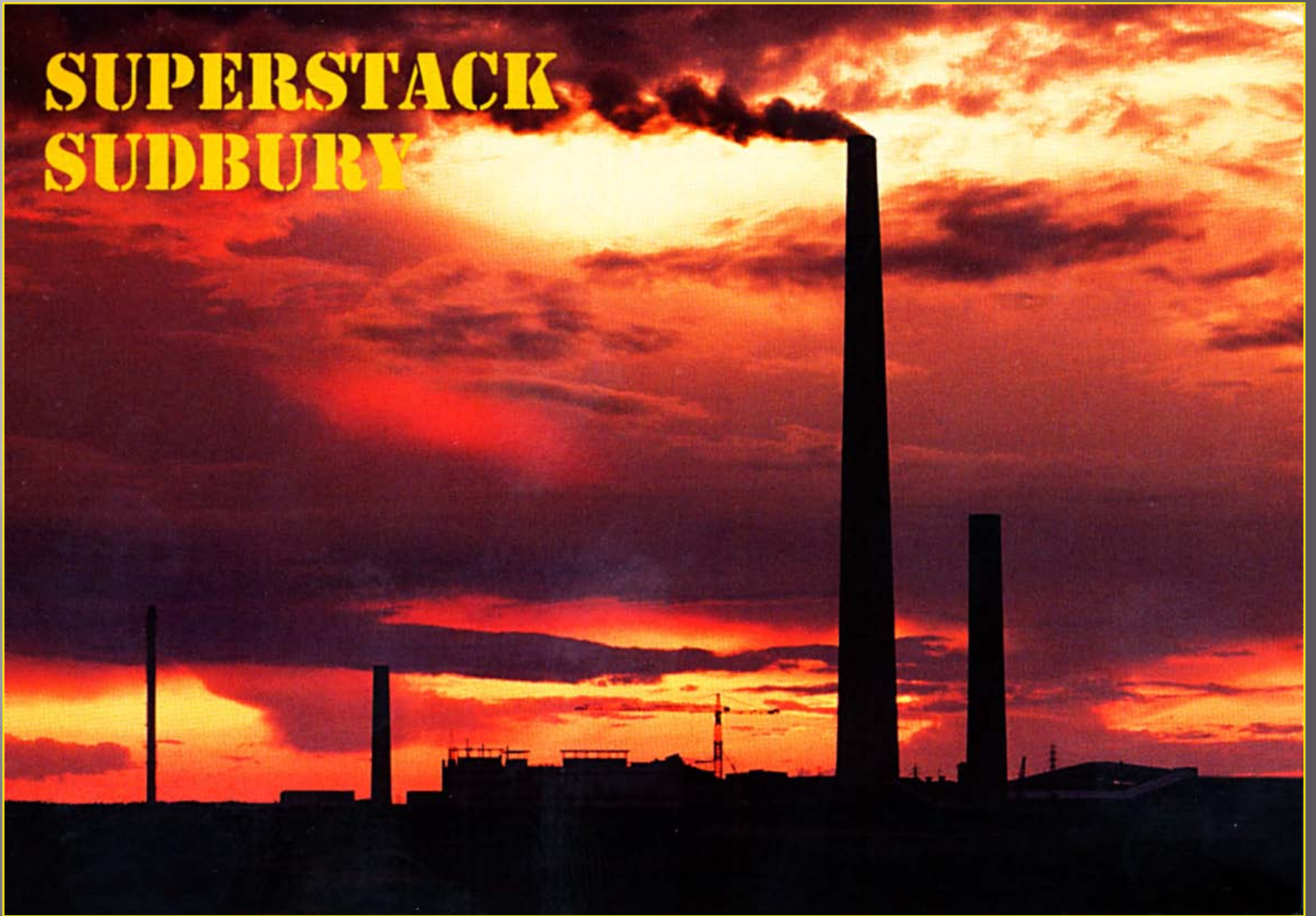
EPIGENETIC MECHANISMS OF NICKEL CARCINOGENESIS

Max Costa

NYU School of Medicine

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE NOV 2010		2. REPORT TYPE		3. DATES COVERED 00-00-2010 to 00-00-2010	
4. TITLE AND SUBTITLE Epigenetic Mechanisms of Nickel Carcinogenesis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) NYU School of Medicine ,550 First Ave,New York,NY,10016				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES Presented at the Depleted Uranium Symposium, held November 4, 2010, at the Armed Forces Radiobiology Research Institute					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 63	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

SUPERSTACK SUDBURY



Nickel Compounds

- ▣ Occupational exposures in Nickel refining industry and environmental exposures from oil and coal burning power plants.
- ▣ Although Ni ions are required for certain enzymes in bacteria and plants (Ureases, Dehydrogenases), No known function in mammals.
- ▣ Certain particulate Ni compounds(Ni_3S_2) that deliver Ni ions into cells, are potently carcinogenic (nasal, lung cancers etc at site of exposure). Not Mutagenic but can induce many diverse types of cancers at the site of exposure and in many different species.
- ▣ Ni ions do not induce or bind to Mt or Ferritin, there is little protection in Human cells for these metal ions. However Ni ions are not very toxic to cells which may allow cancer cells to arise with epigenetic and genetic alterations

Facultative
Phagocytosis

+ +
+ +
+
Amorphous
NiS

- -
- -
-
Crystalline
NiS
 Ni_3S_2

Ni^{2+}
less
Uptake

DMT-1

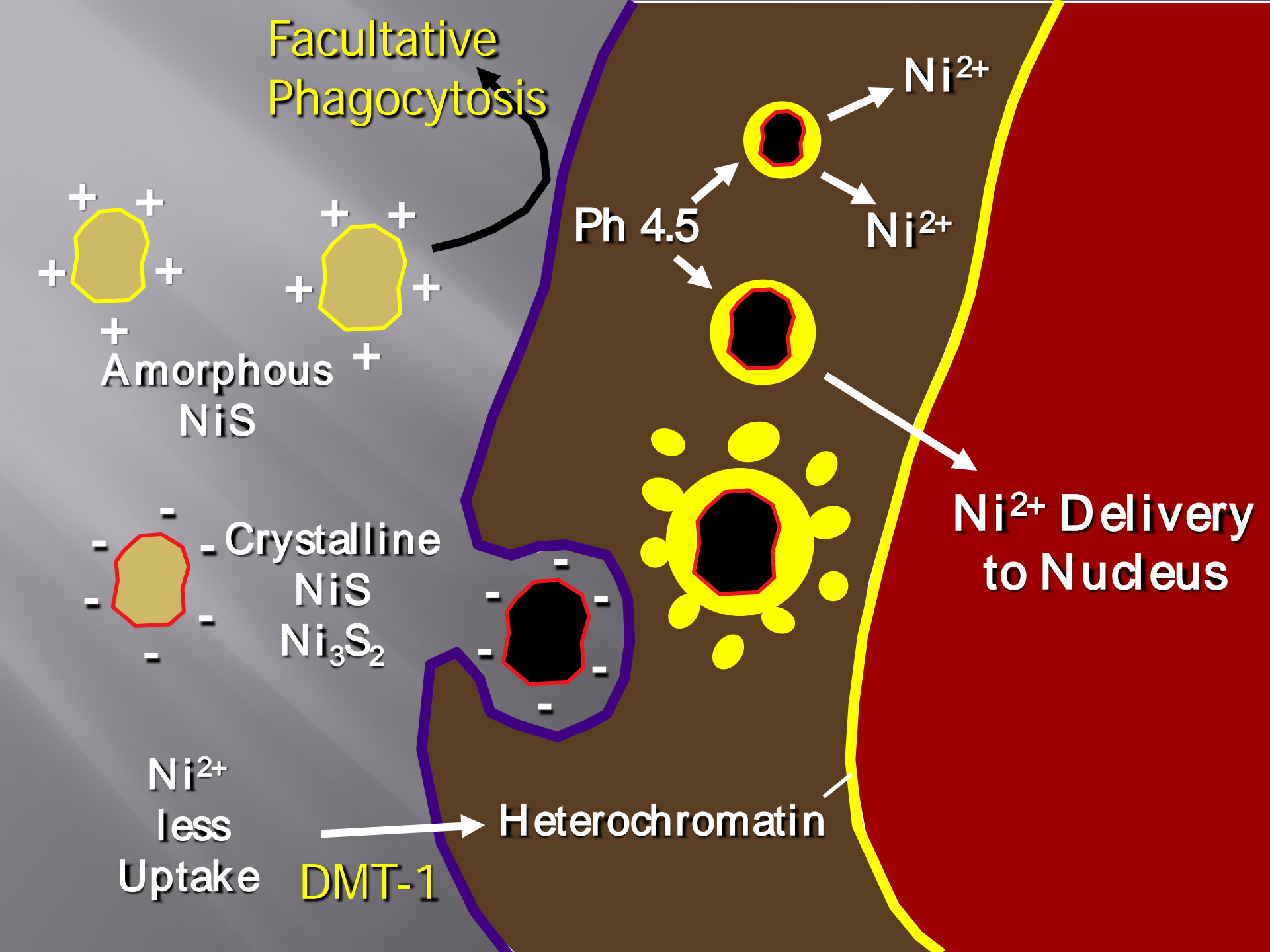
Heterochromatin

Ph 4.5

Ni^{2+}

Ni^{2+}

Ni^{2+} Delivery
to Nucleus



Potential Intracellular Concentration of a Phagocytized Crystalline NiS Particle^a

Mean particle diameter used in calculation (μm)	Approximate NiS cellular concentration ^b (M)
1.45	0.25
4.00	4.75

^aCell volume was determined in CHO cells with a Coulter counter-particle size analyzer and log range expander.

^bCell volume, $393.5, \mu\text{m}^3$; density of NiS, $5.5\text{g}/\text{cm}^3$; particles assumed spherical.

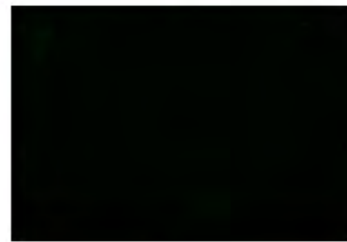
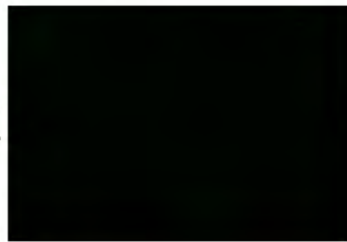
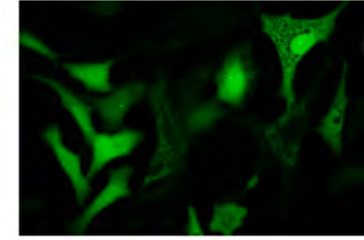
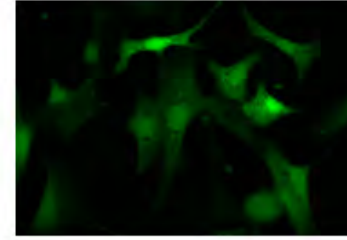
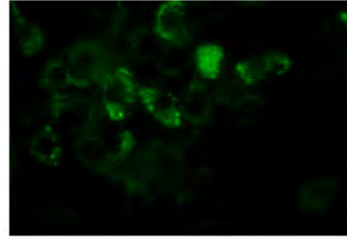
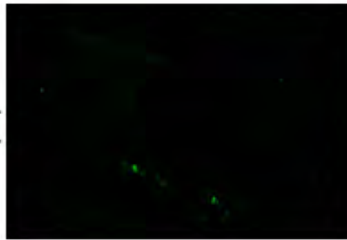
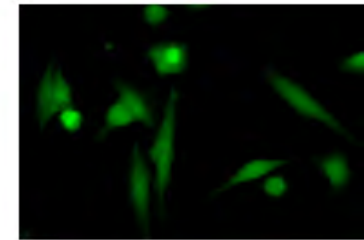
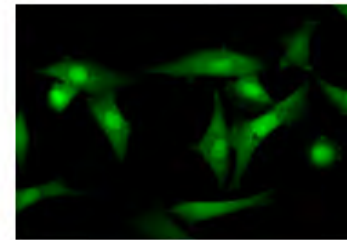
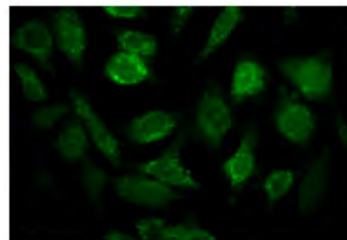
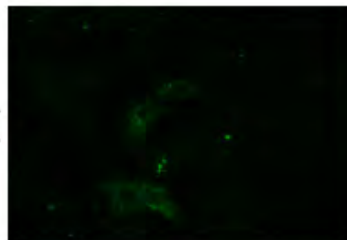
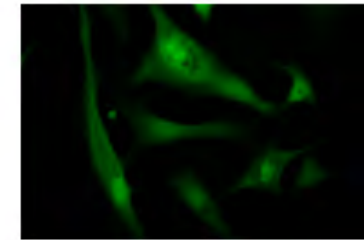
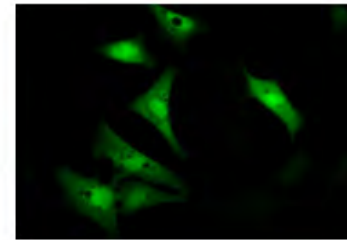
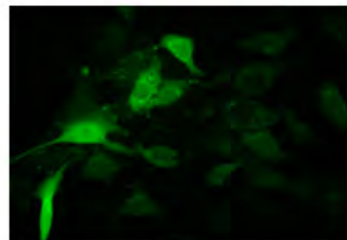
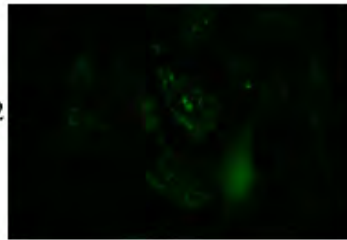
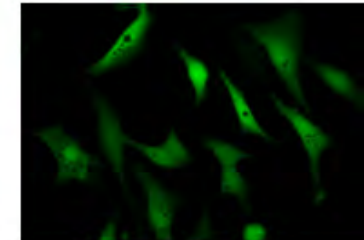
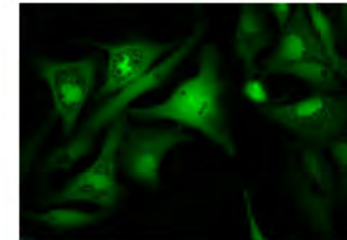
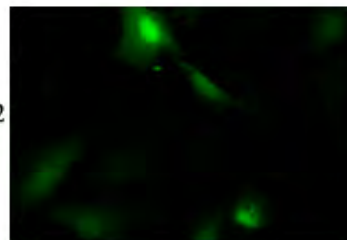
8h

24h

48h

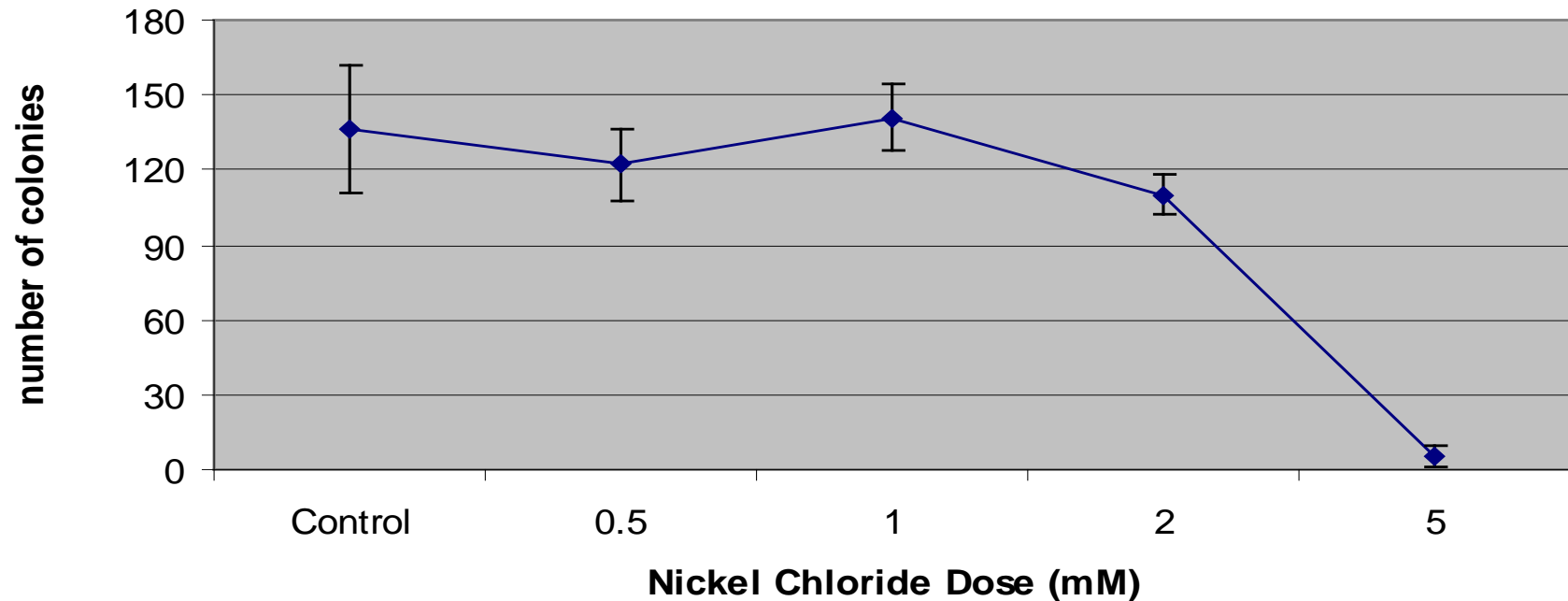
72h

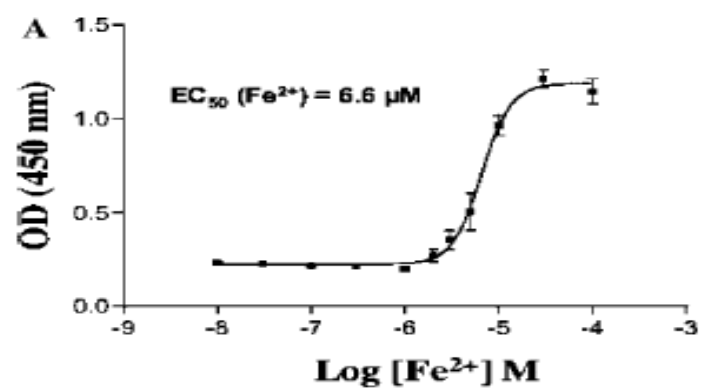
Control

NiCl₂ 0.5mMNiCl₂ 1.0mMNi₃S₂ 0.5μg/cm²Ni₃S₂ 1.0μg/cm²

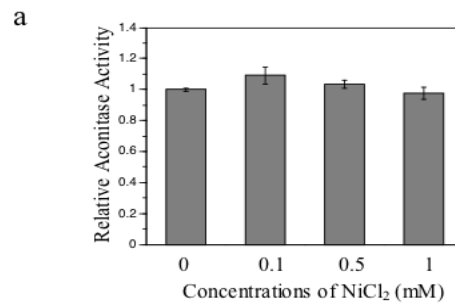
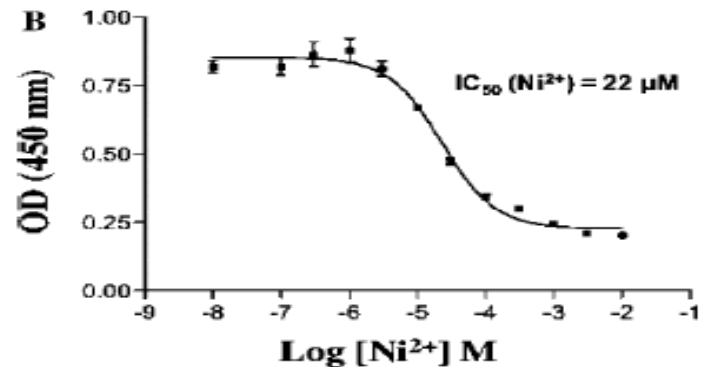
Effect of 24 hr exposure of A549 Cells to NiCl_2 On Cell Colony Formation

Cell Colony Formation After Ni Treatment

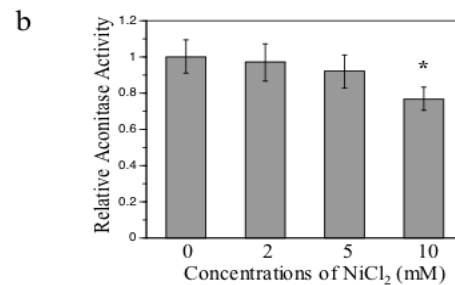




Prolyl
hydrox



aconitase



Periodic Table

12	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8 VIII	9 VIII	10 VIII	11 IB	12 IIB	13 IIIA
20 Sc 21 44.955910 13 3+ Scandium	22 Ti 22 47.88 15 4+ Titanium	23 V 23 50.9415 16 5+ Vanadium	24 Cr 24 51.9961 16 3+ Chromium	25 Mn 25 54.93805 15 2+ Manganese	26 Fe 26 55.847 18 3+ Iron	27 Co 27 58.9332 18 2+ Cobalt	28 Ni 28 58.6934 18 2+ Nickel	29 Cu 29 63.546 19 2+ Copper	30 Zn 30 65.39 16 2+ Zinc	31 Ga 31 69.723 15 3+ Gallium	
38 Y 39 88.90585 13 3+ Yttrium	40 Zr 40 91.224 14 4+ Zirconium	41 Nb 41 92.90638 16 5+ Niobium	42 Mo 42 95.94 18 6+ Molybdenum	43 Tc 43 98.9063 19 7+ Technetium	44 Ru 44 101.57 22 3+4+ Ruthenium	45 Rh 45 102.9055 22 3+ Rhodium	46 Pd 46 106.42 22 2+ Palladium	47 Ag 47 107.8682 19 1+ Silver	48 Cd 48 112.411 17 2+ Cadmium	49 In 49 114.818 15 3+ Indium	
56 La 57 138.9055 11 3+ Lanthanum	72 Hf 72 178.49 13 4+ Hafnium	73 Ta 73 180.9479 15 5+ Tantalum	74 W 74 183.85 17 6+ Tungsten	75 Re 75 186.207 19 7+ Rhenium	76 Os 76 190.2 22 4+ Osmium	77 Ir 77 192.22 22 4+ Iridium	78 Pt 78 195.08 22 4+ Platinum	79 Au 79 196.96654 24 3+ Gold	80 Hg 80 200.59 19 2+ Mercury	81 Tl 81 204.38 15 3+ Thallium	
88 Ac 89 227.0278 11 3+ Actinium	104 Rf 104 261.11 - - Rutherfordium	105 Db 105 262.11 - - Dubnium	106 Sg 106 263.12 - - Seaborgium	107 Bh 107 262.12 - - Bohrium	108 Hs 108 264 - - Hassium	109 Mt 109 266.1378 - - Meitnerium	110 Uun 110 269 - - Ununnilium	111 Uuu 111 272 - - Unununium	112 Uu 112 277 - - Ununbium	113 Uuh 113 288 - - Ununtrium	

Examples of Oxoglutarate Superfamily of Dioxygenases Enzymes in Humans (more than 100 across evol. phyla)

Prolyl Hydroxylases (collagen, HIF-dependent)

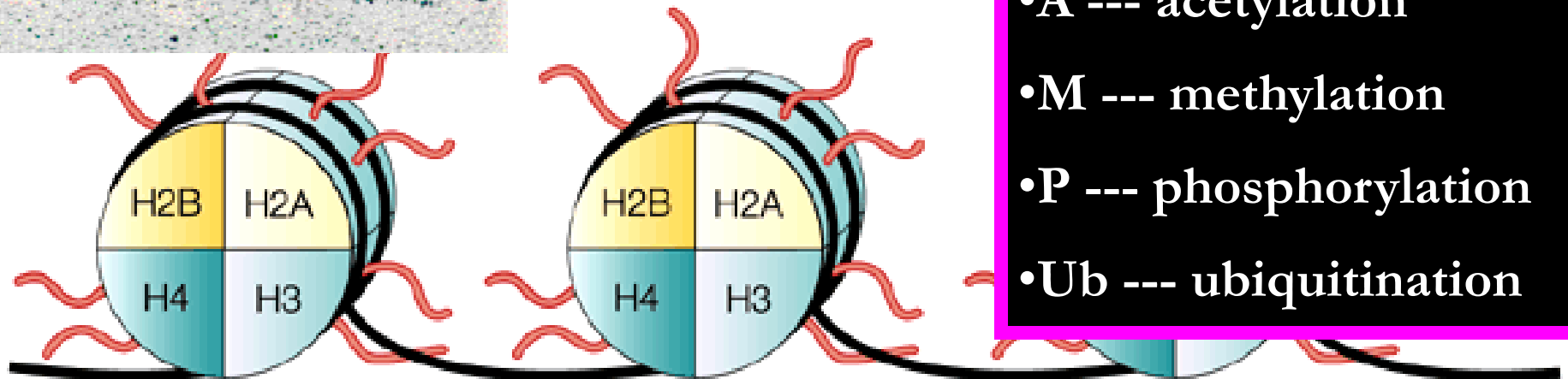
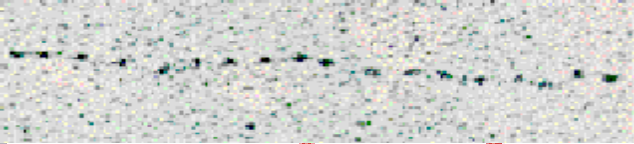
Asparagine Hydroxylases (FIH)

Alk b DNA repair enzymes (1-meA, 3-meC, ABH2 and ABH3)

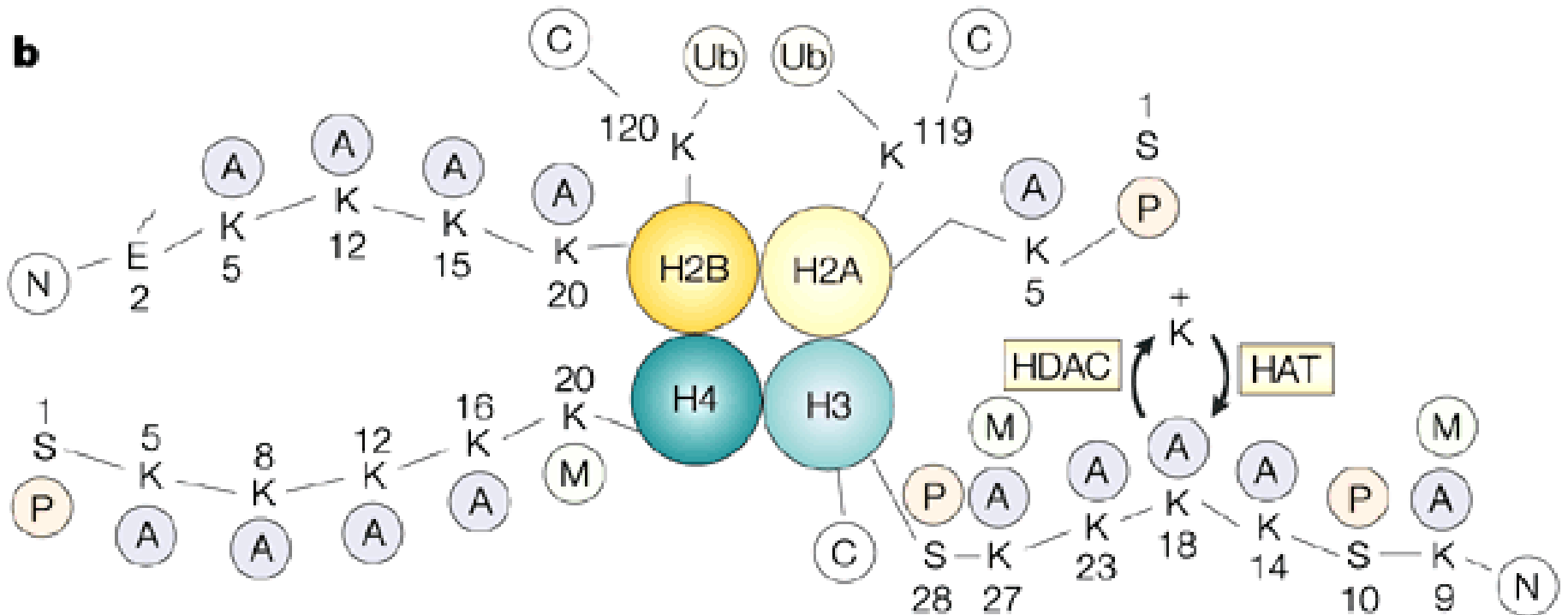
FTO (3-meT, 3meC, overexpression weight gain, Type 2 Diabetes)

Histone Lysine Demethylases

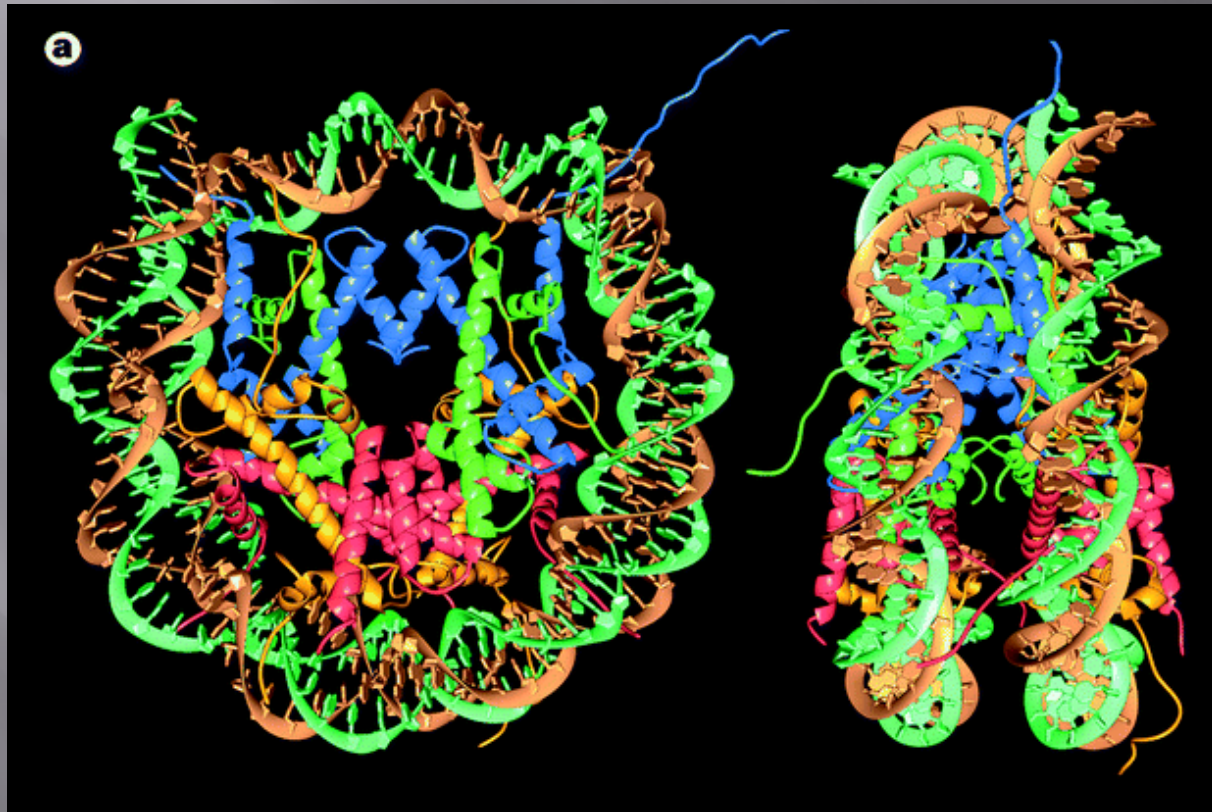
(only use of Ascorbic Acid in our bodies)



- A --- acetylation
- M --- methylation
- P --- phosphorylation
- Ub --- ubiquitination



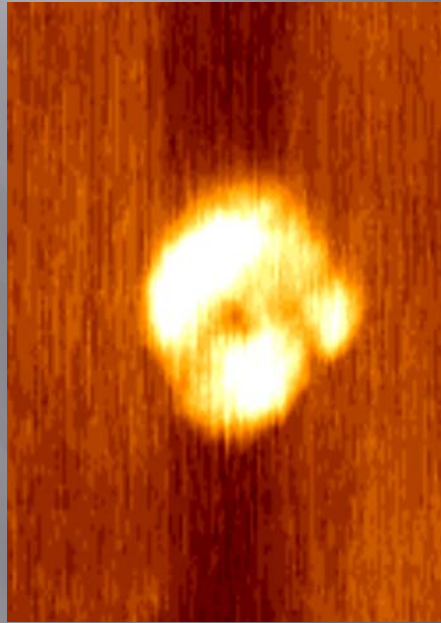
Structure of Nucleosome



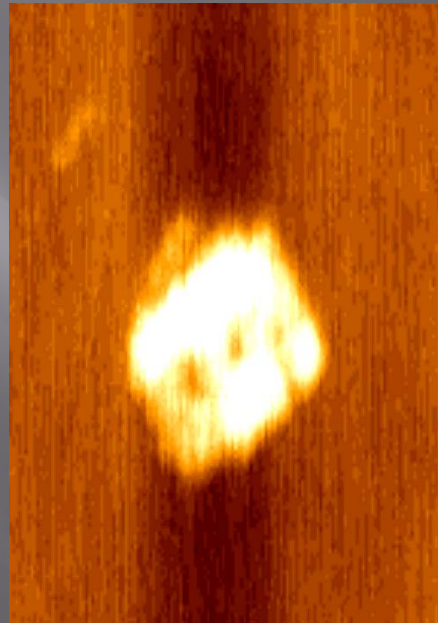
Luger K et al. Nature, **389**:251

Blue: H3; green: H4; yellow: H2A; red: H2B

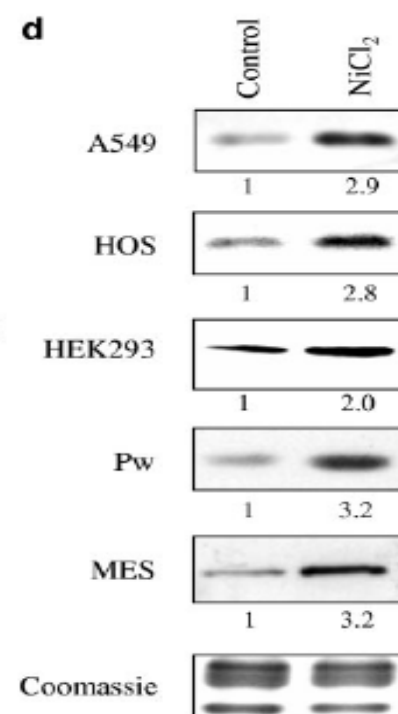
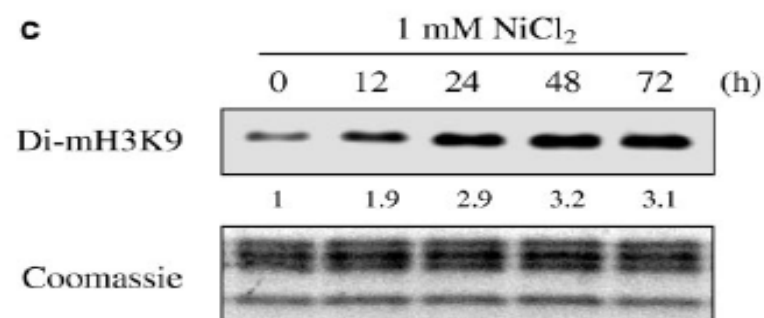
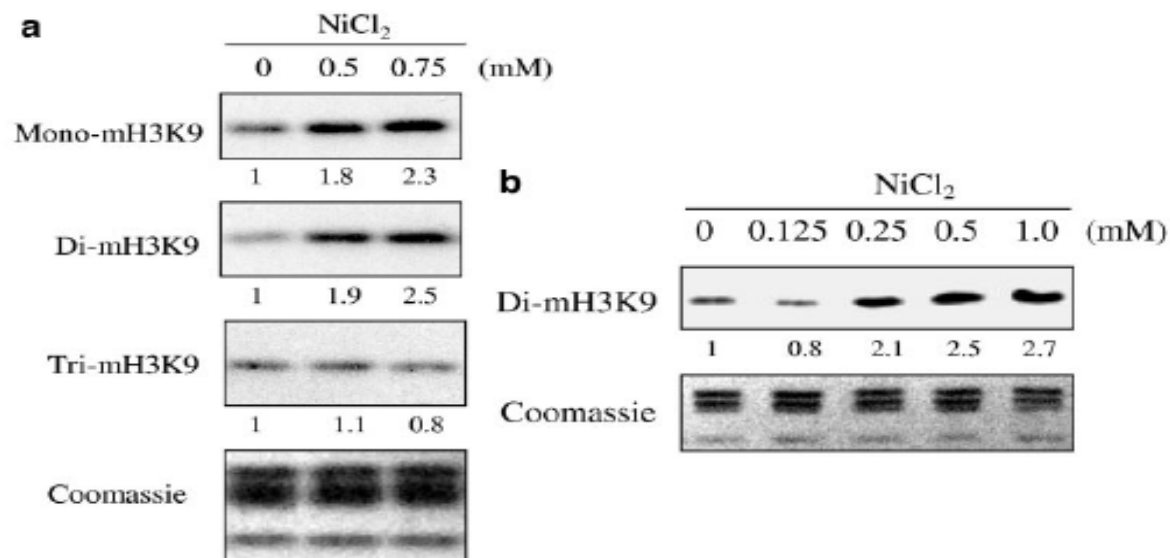
Atomic Force Microscopy View of Chicken Erythrocyte Single Nucleosomes



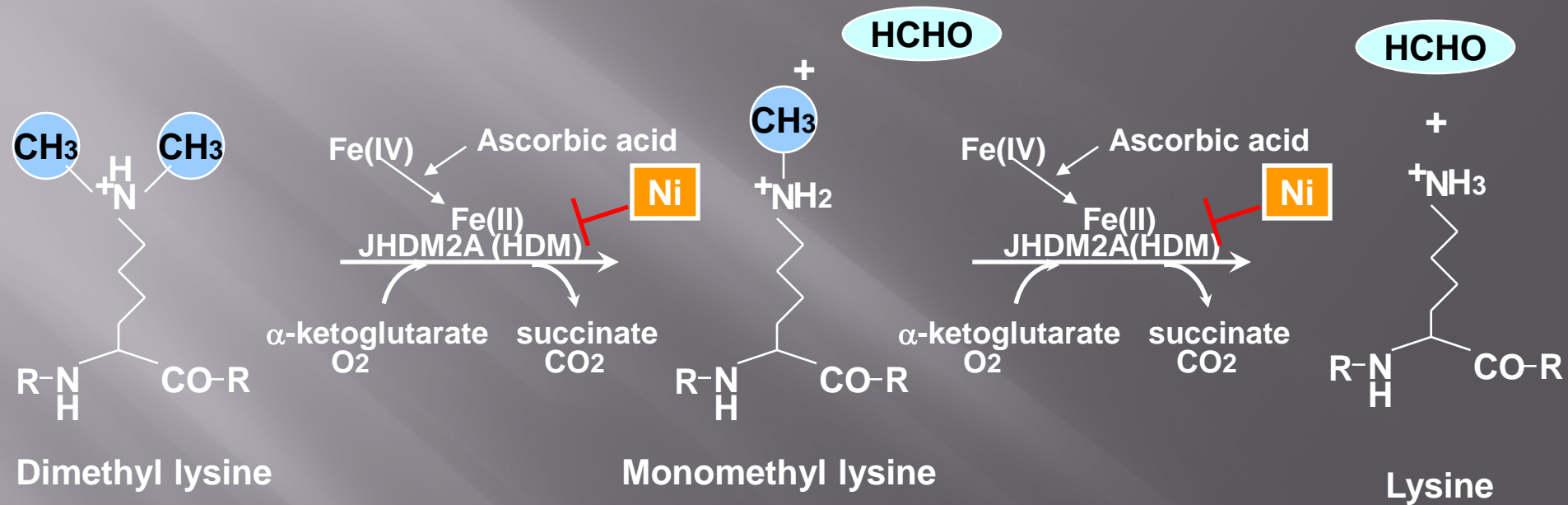
end on
view



face
view




JMJD1A (Histone 3 Lysine 9 Di and Mono Demethylase JHDM2A)



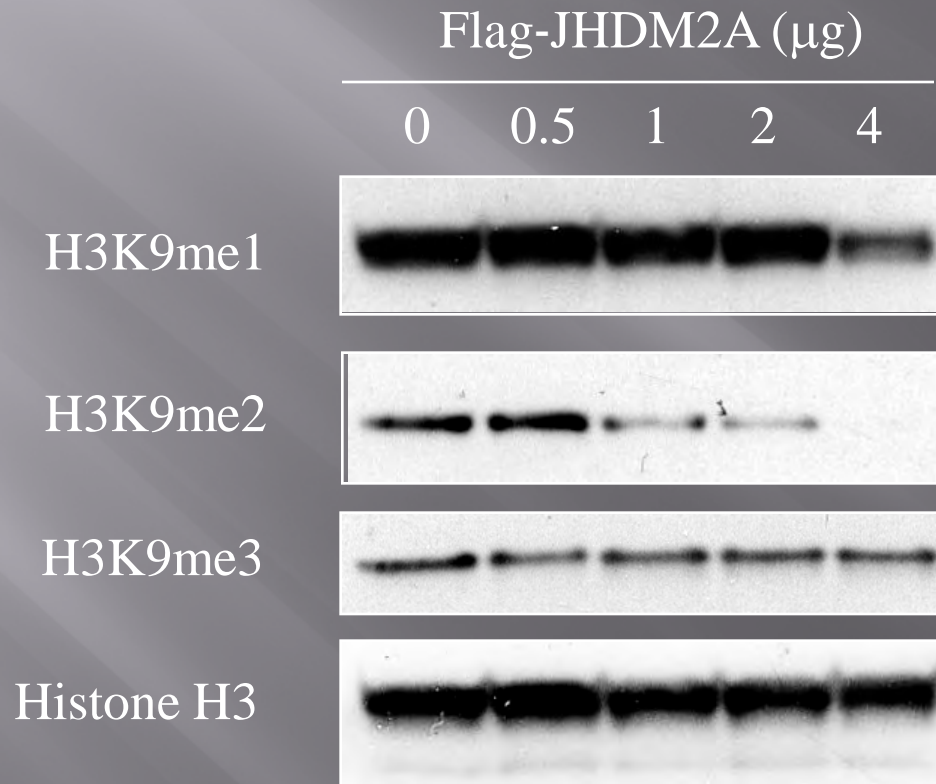
H3K9me²

Human histone demethylases (HDMs)

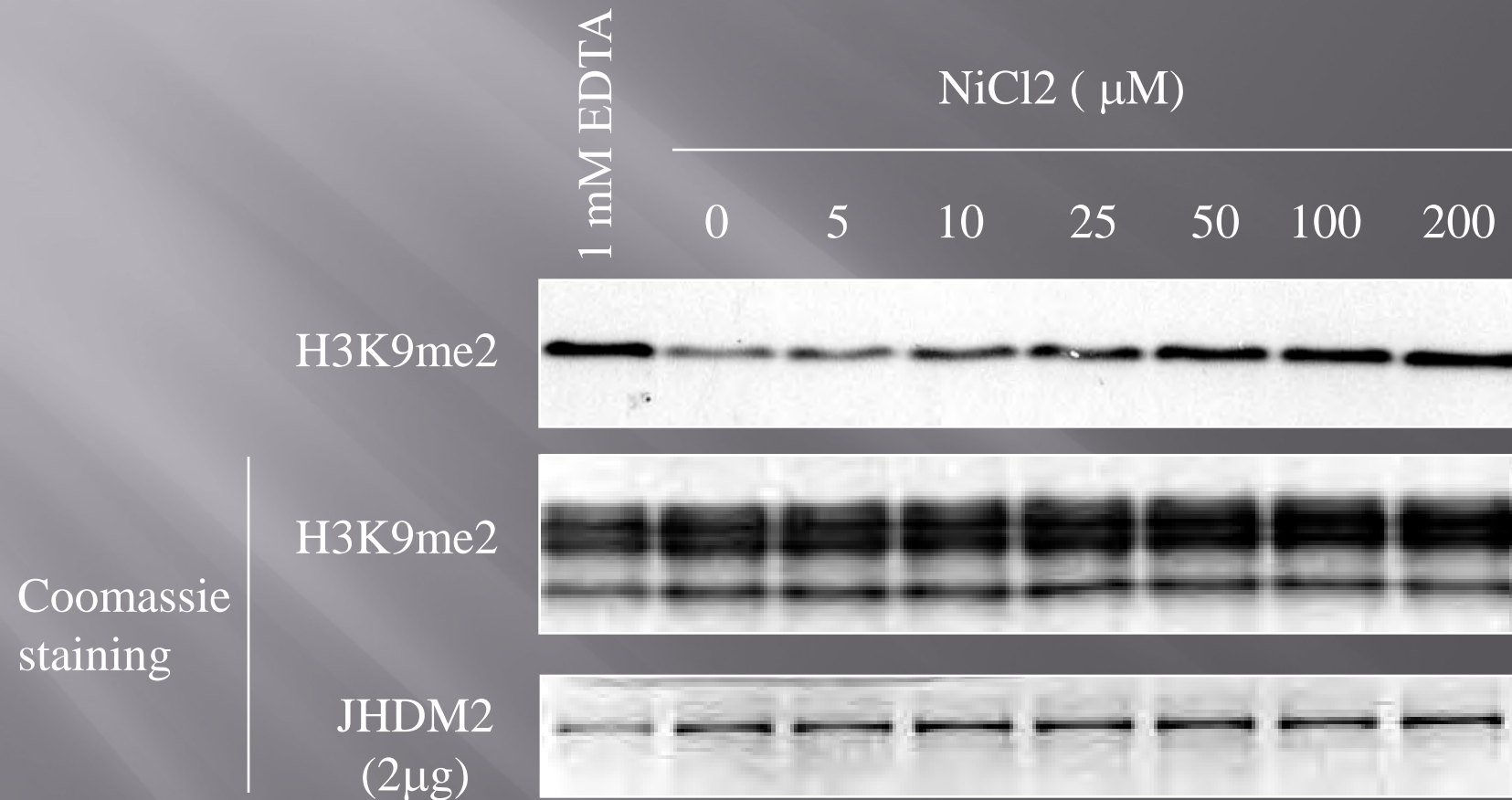
name	substrate
PADI4	R on H3, H4 (No Fe)
LSD1	H3K4 (No Fe)
JHDM1	H3K9, H3K36 (di-), Fe dep
JHDM2A /JMJD1A	H3K9 (mono-, di-)
JHDM3A /JMJD2A	H3K9 (tri-), H3K36 (tri-)
GASC1/JMJD2C	H3K9 (di-, tri-)
JARID-1 family (4 members Jmjc dom)	H3K4 (Tri)



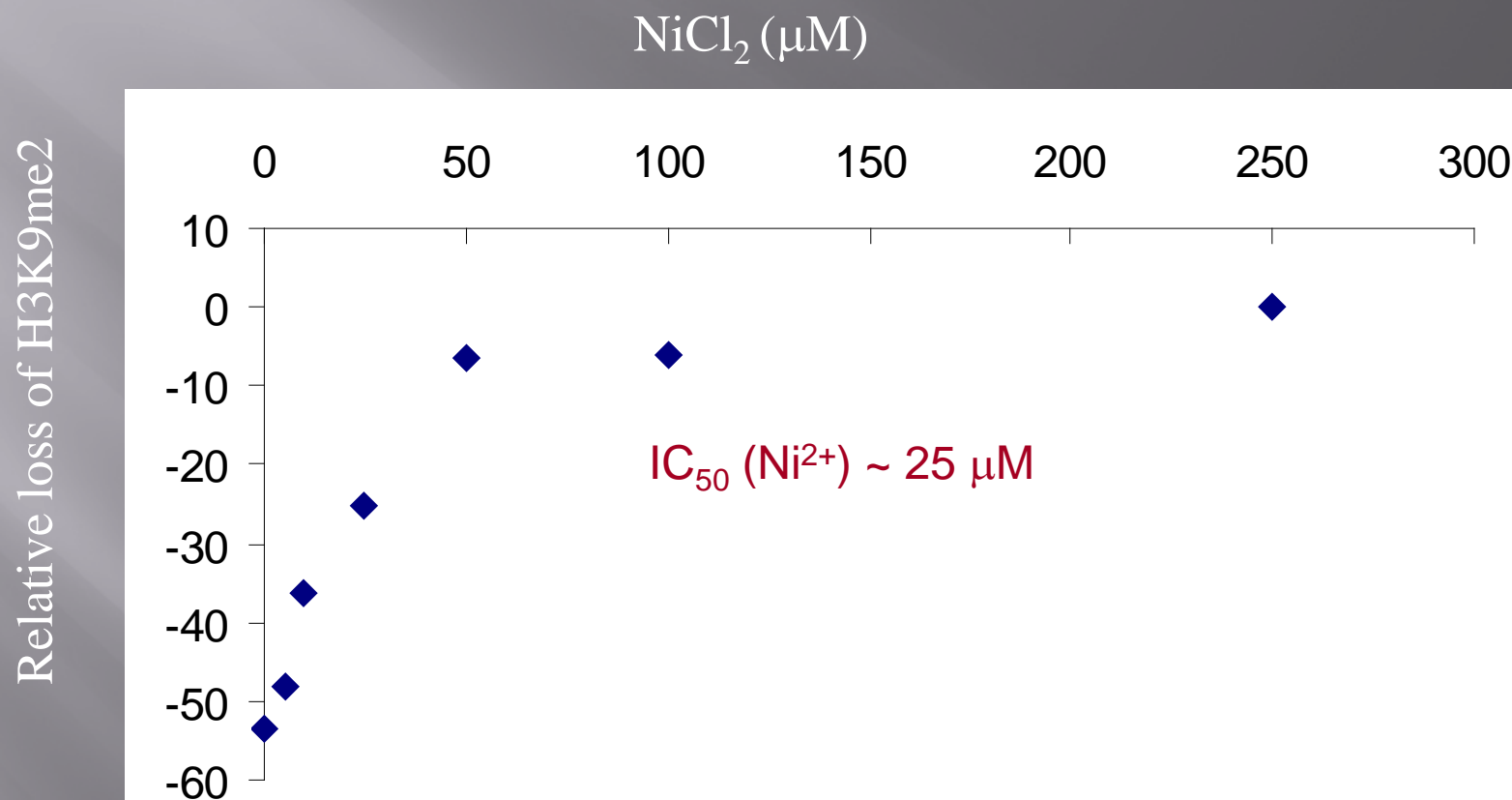
In vitro H3K9 Demethylation by Flag-JHDM2A recombinant protein



Dose-dependent inactivation of JHDM2A by Ni ions (Ni ions added last)

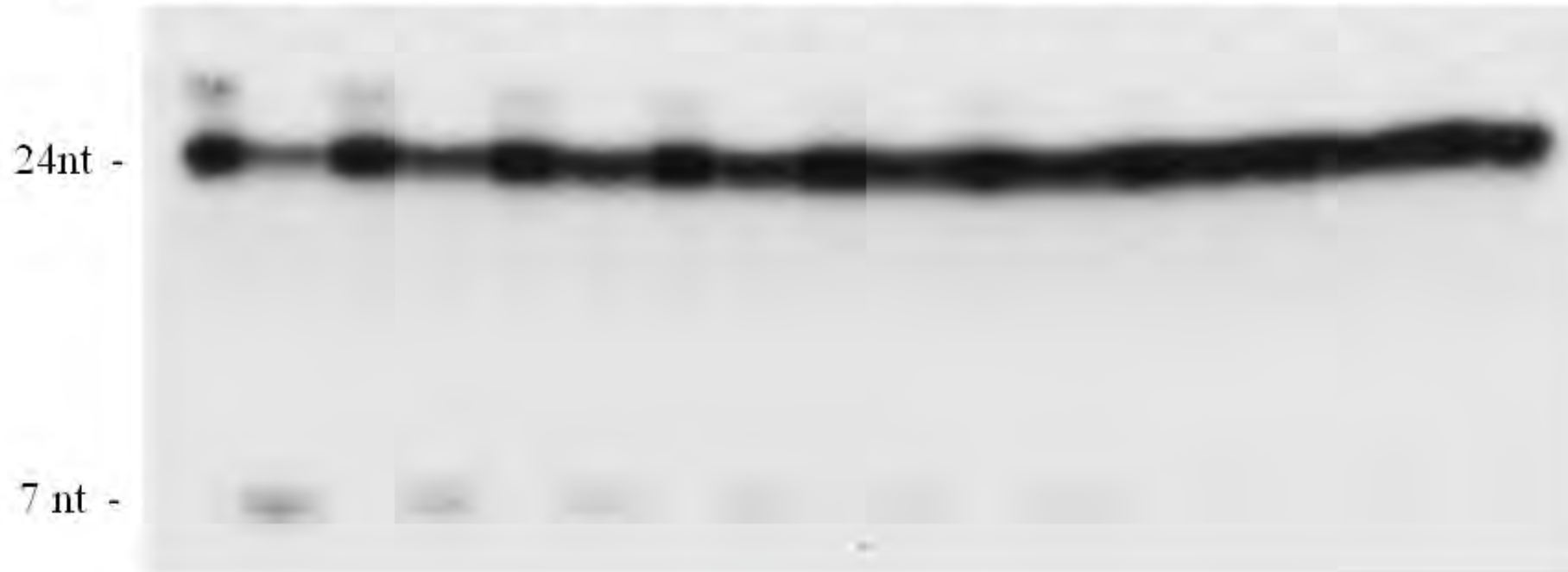


Inactivation of JHDM2A by Ni ions



Dose-dependent inactivation of ABH2 by Ni ions

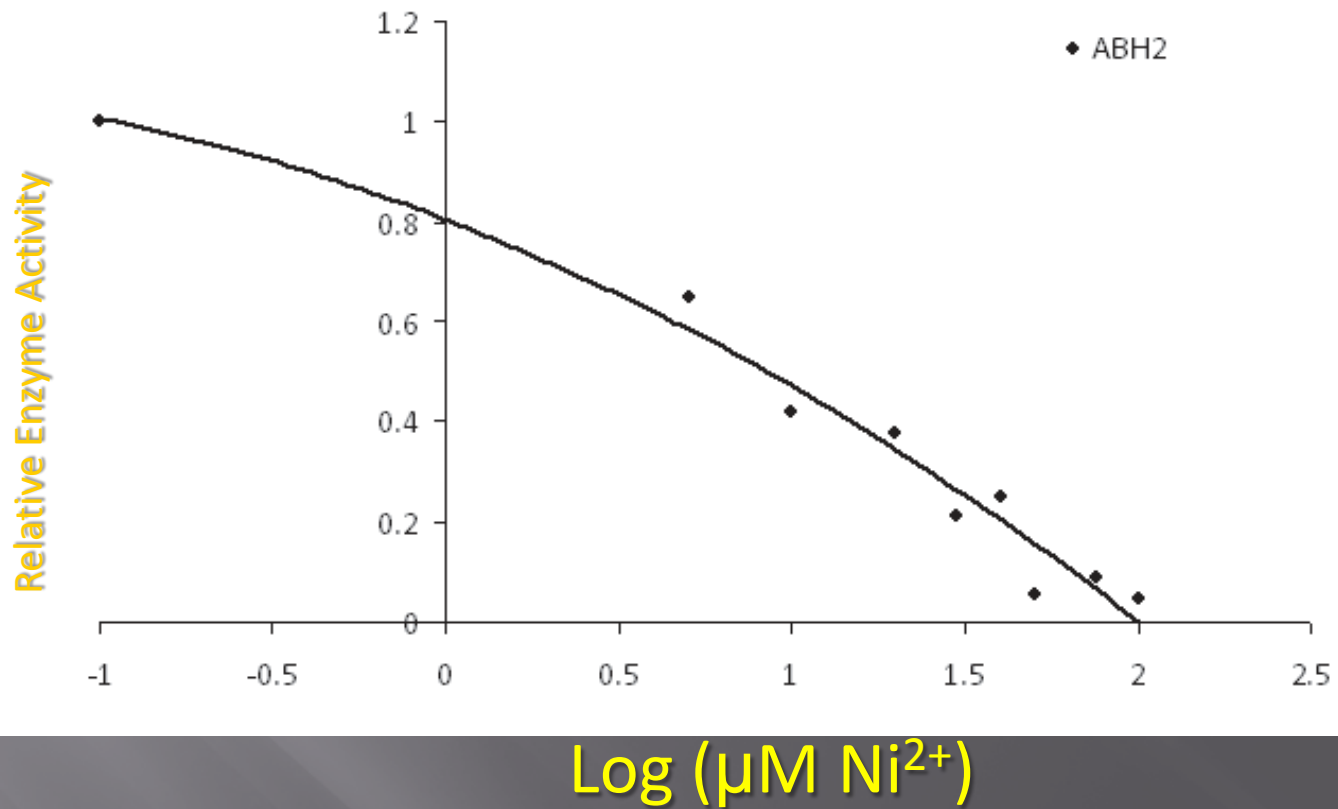
NiCl ₂ (μM)	0		5		10		20		30		40		50		75		100	
HpaII	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+



Oligo 3-meC: 5'-³²PAAA GCA ^{3-me}CCG GTC GAA AAA GCG AAA-3'

30 min 1 μg ABH2 demethylation → Annealing → 1 h HpaII cutting

Data Quantification

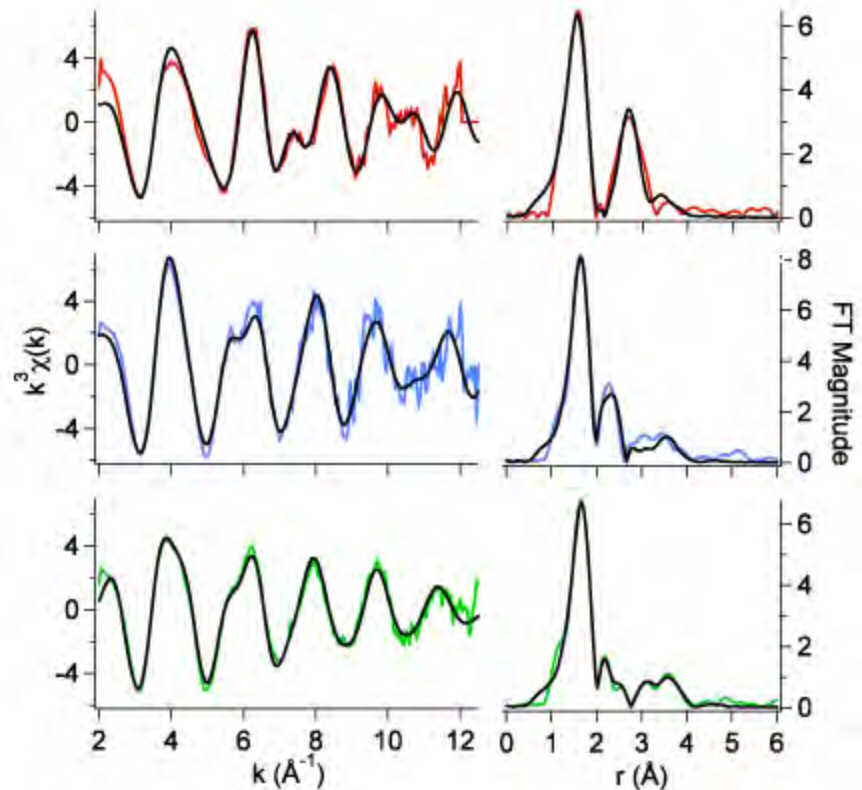
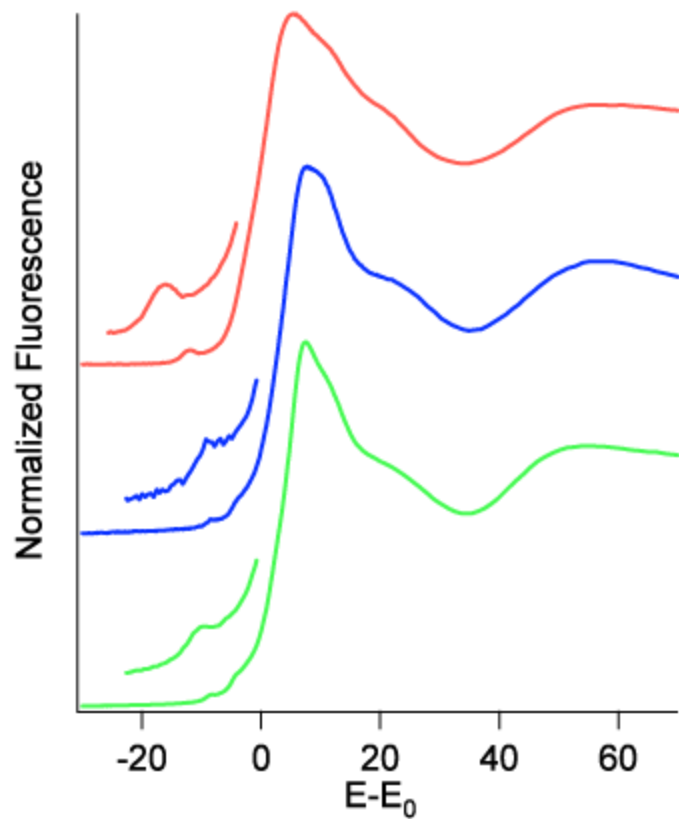


Kd for Ni and Fe binding to ABH2 (DNA Demethyl)

Fe=4.5uM

Ni=1.7uM

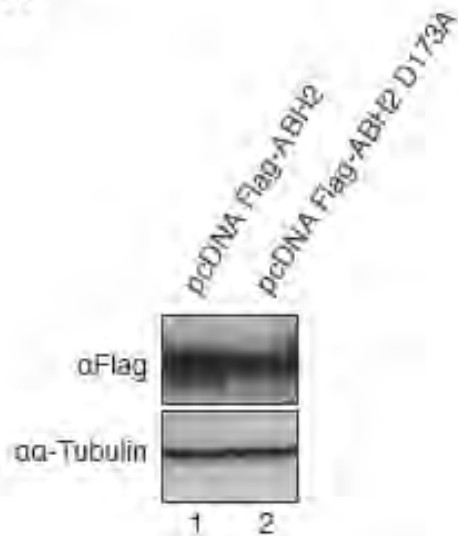
Giri and Maroney, Dept Chem, Univ of Mass



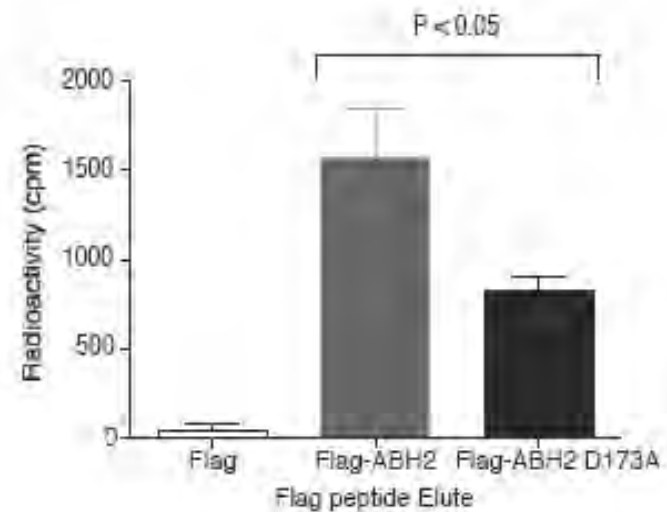
XAS analysis of nickel binding to ABH2. A). *K*-Edge XANES spectra for Fe-ABH2 (red), Ni-ABH2 (blue) and Ni-ABH2 + 2-oxoglutarate (green). Inserts: Expansions of the preedge XANES region showing peaks associated with $1s \rightarrow 3d$ electronic transitions. B). Unfiltered, k^3 -weighted EXAFS spectra (colored lines, red = Fe-ABH2, blue = Ni-ABH2 and green = Ni-ABH2 + 2-oxoglutarate) and best fits from Table 1 (black lines). Left: k -space spectra and fits. Right: FT-data and fits.

Ni ion binding to ABH2 In Intact Cells

A



B



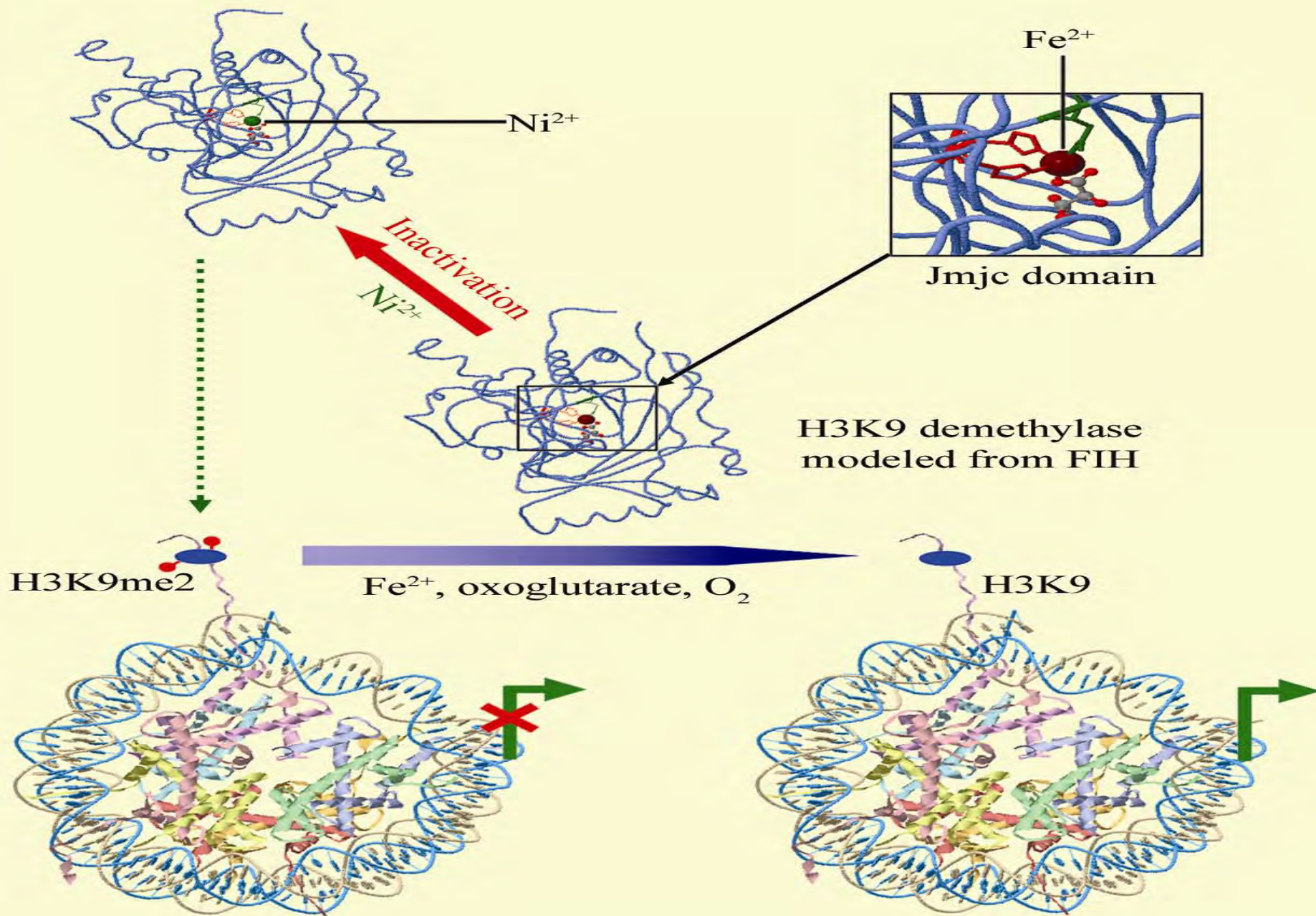
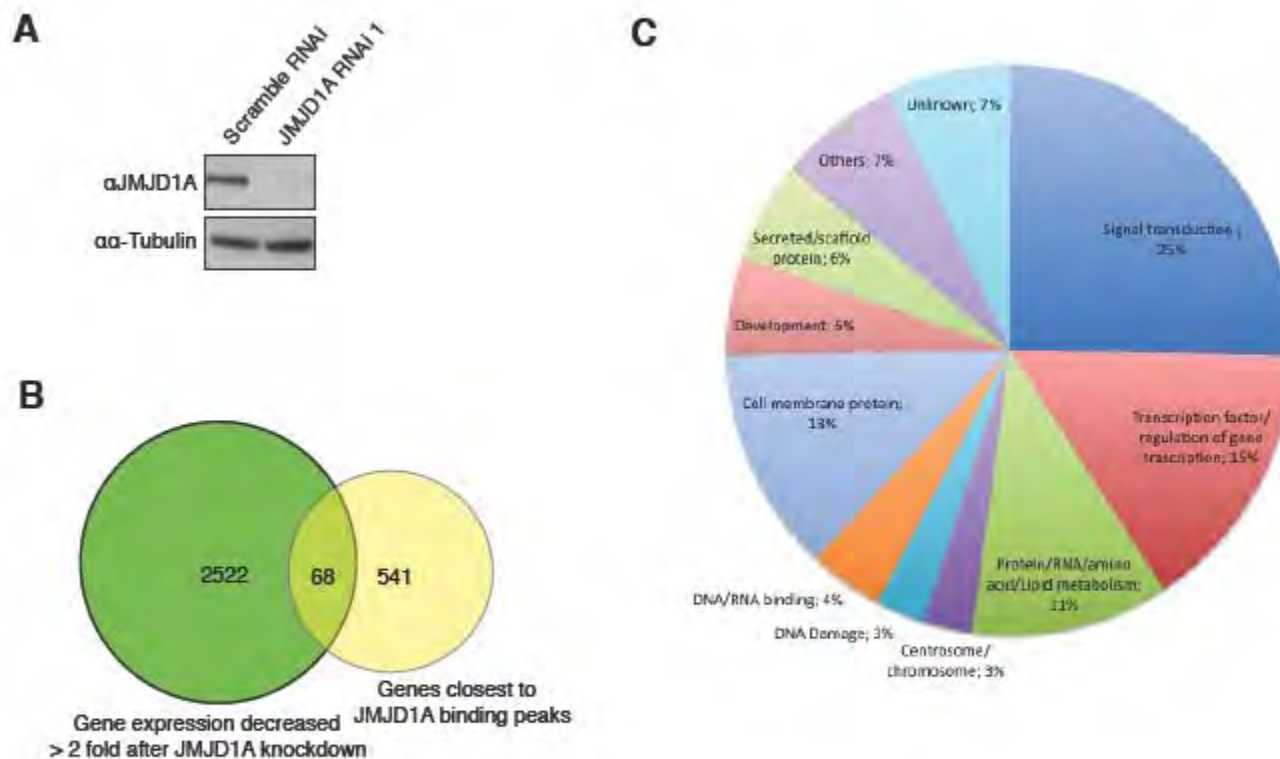
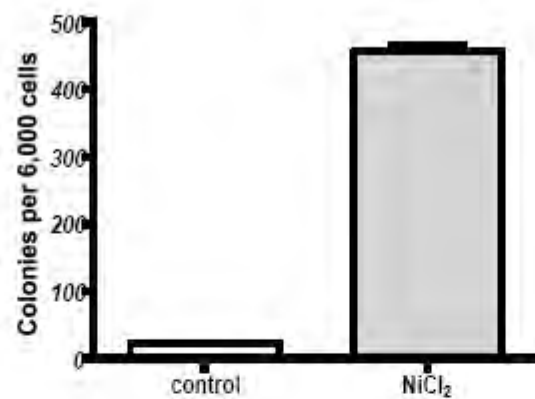
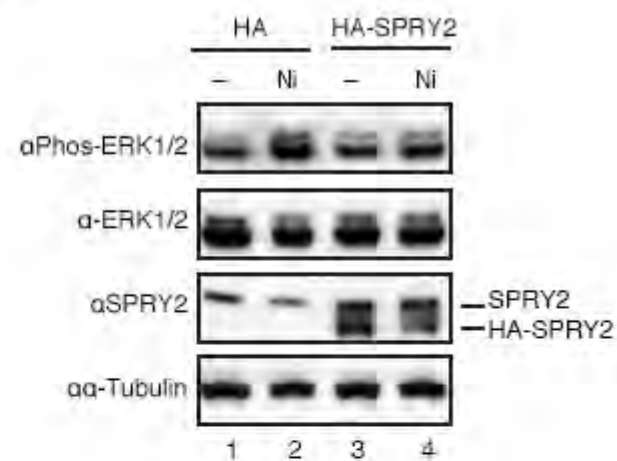
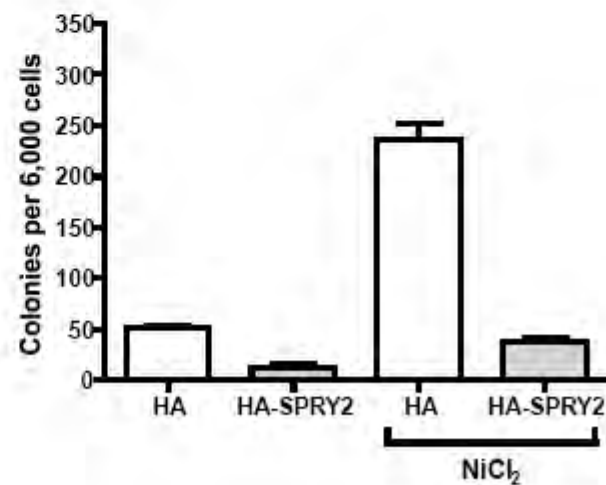
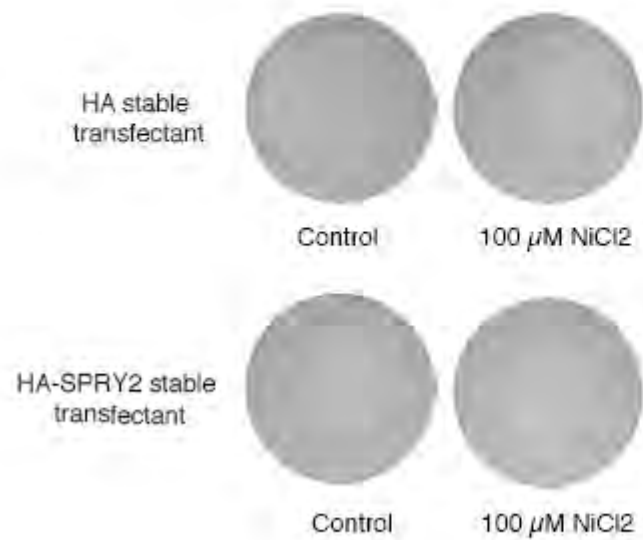


Figure 1



SPRY2 and JHMD2A

- ▣ JHMD2A binds and activates SPRY2 transcription.
- ▣ SPRY2 is epigenetically silenced with H3K9 dimethylation marks in it's promoter in Nickel treated and transformed BEAS2B cells.
- ▣ SPRY2 inhibits growth factor signaling in cells and is a tumor suppressor.

D**E****F**

Heat Maps of Genes Changed in Nickel-transformed Cells

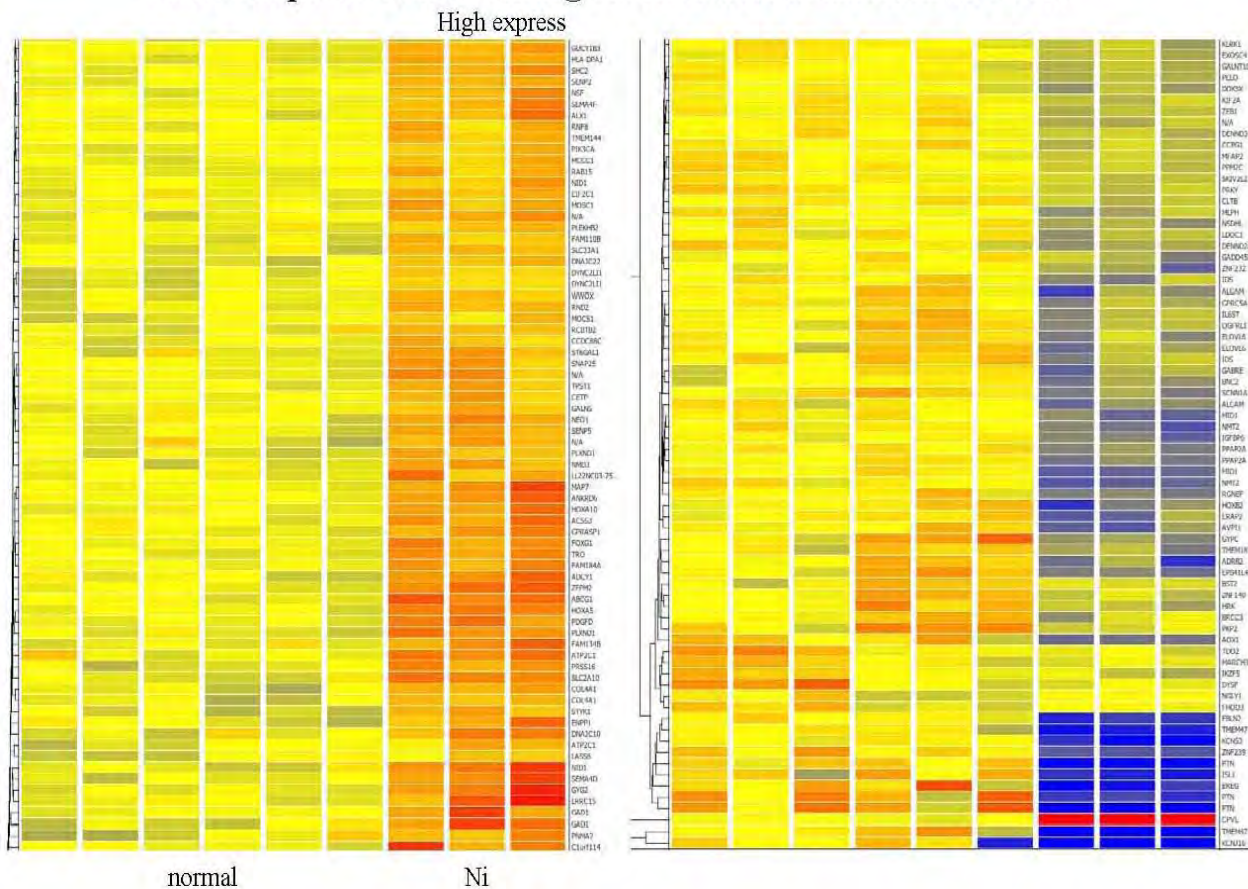


Figure 12. Affymetrix gene expression array heat maps from non-tumorigenic normal BEAS2B cell clones and NiCl₂ transformed BEAS-2B cells. BEAS2B cells were exposed to NiCl₂ at 100uM concentration for 4 weeks and then either unexposed or exposed BEAS2B were plated to proliferate in soft agar. Shown in the figure are Affymetrix arrays from spontaneously derived small agar colonies from untreated cells (first 6 lanes) or large agar colonies from nickel-treated cells. Colonies were picked from the agar and Affymetrix gene expression was determined directly from these agar colonies. The left panel compares the most unregulated genes while the right panels compares the most down regulated genes based upon the nickel transformed clones.

W

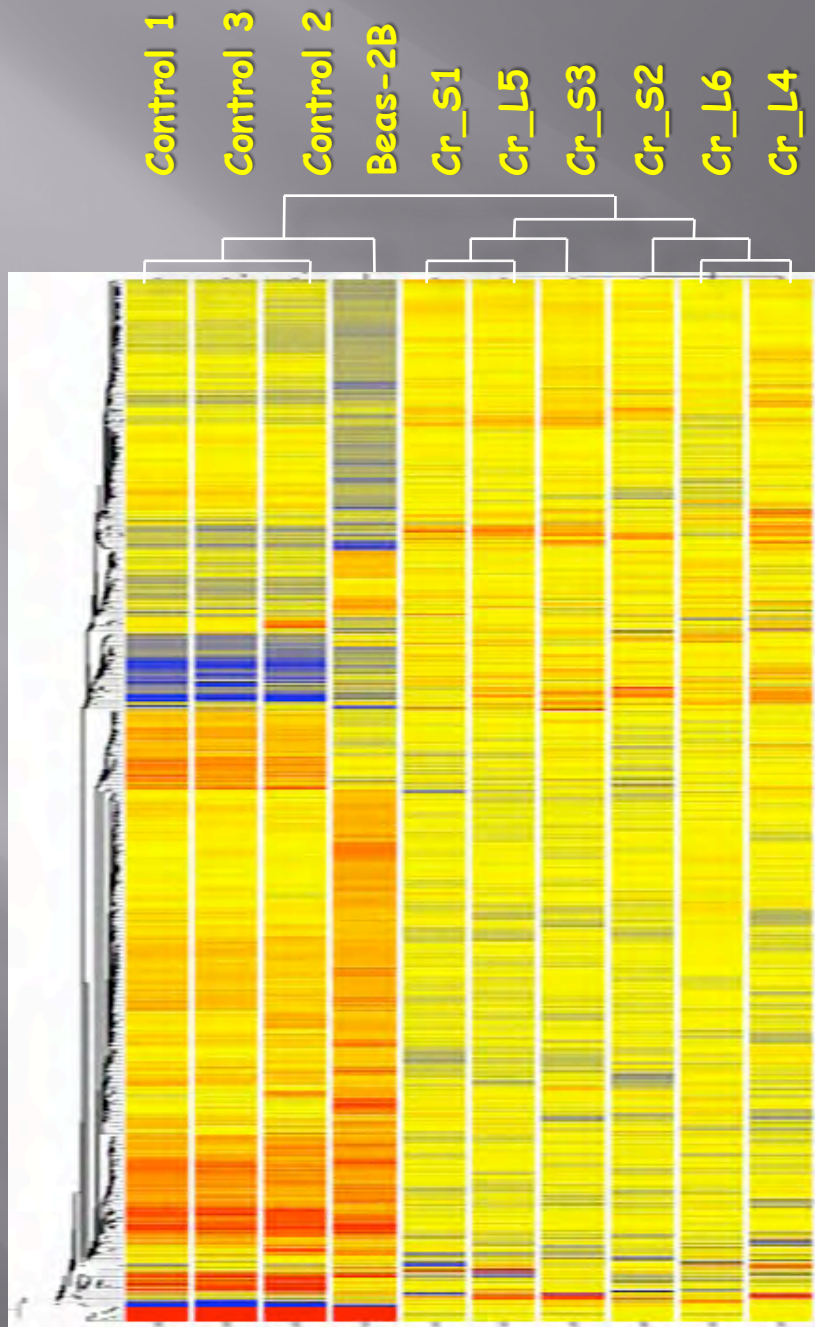
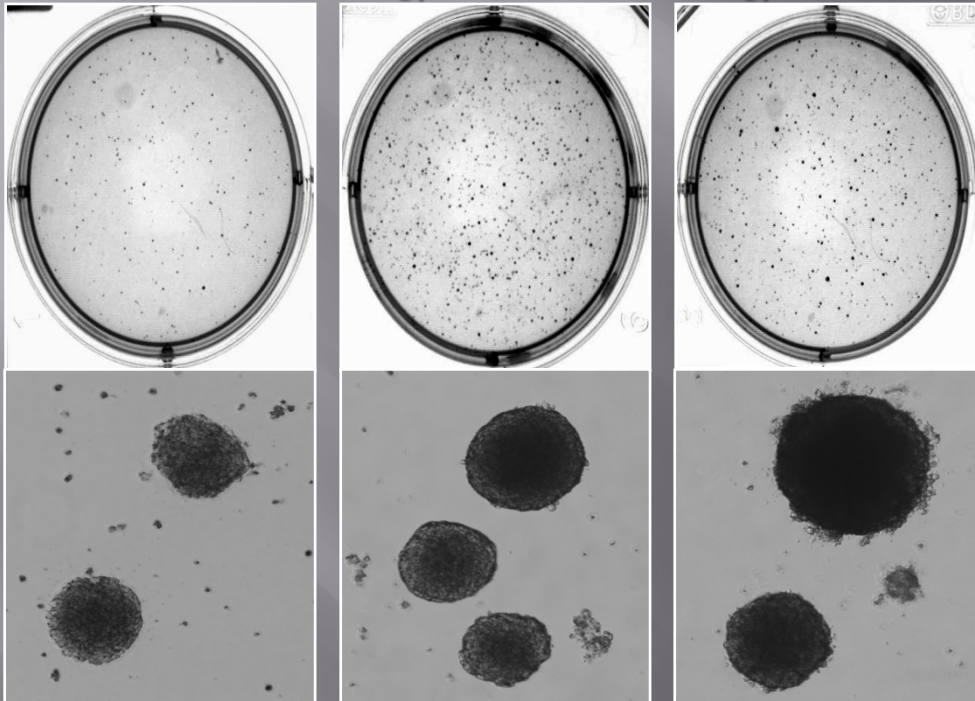
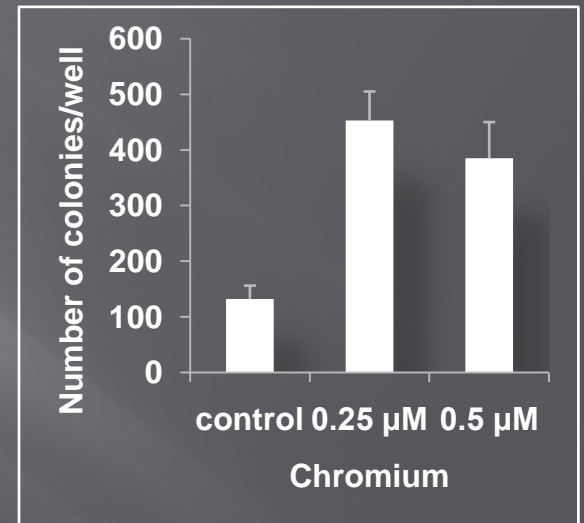
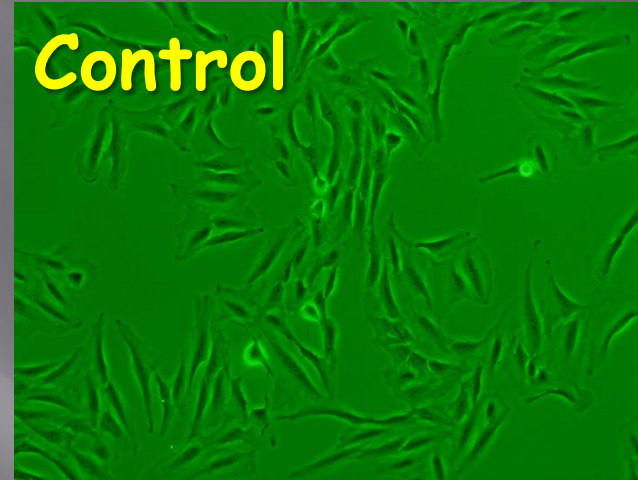
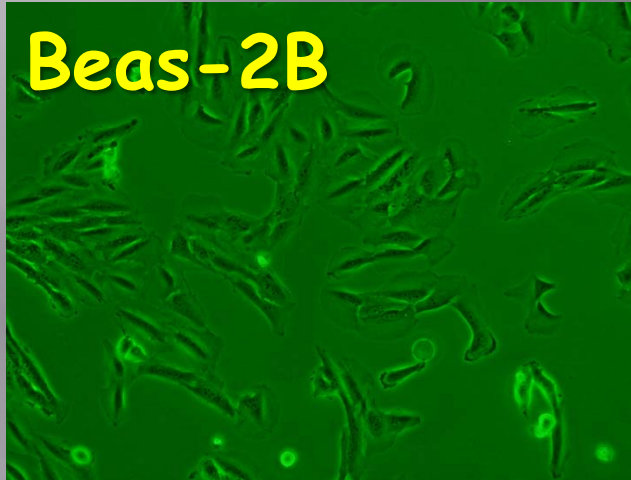


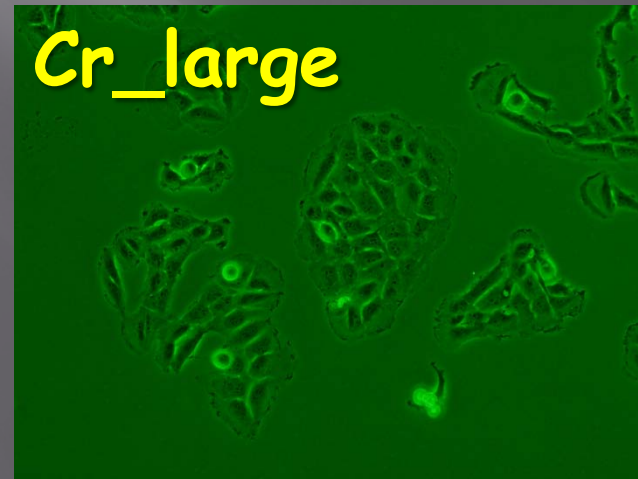
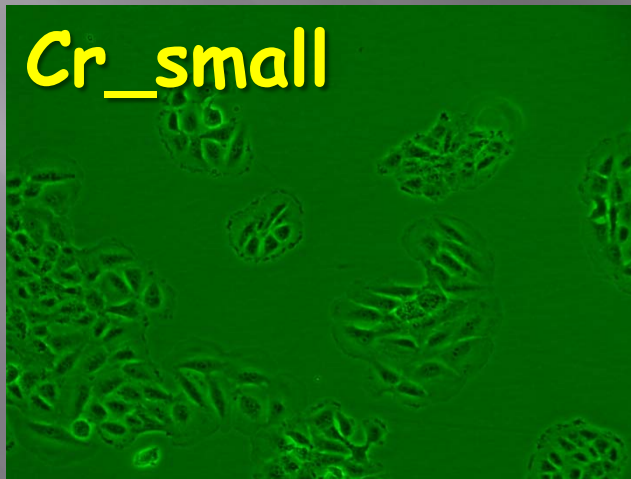
Figure 5

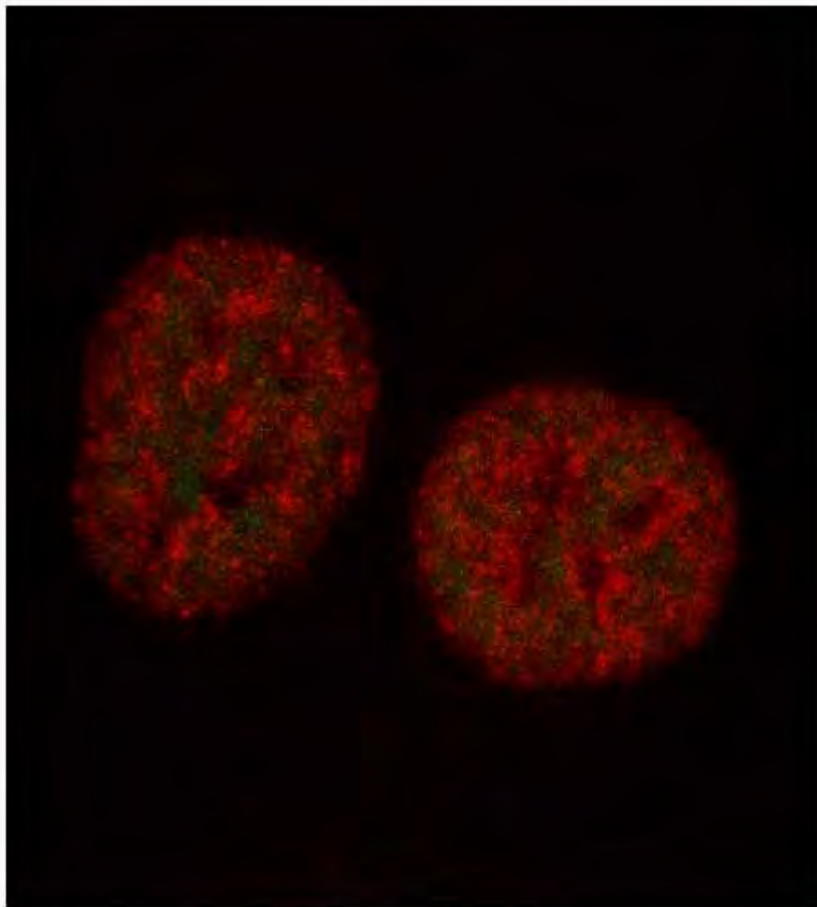
A**Control****0.25 μ M
Cr****0.5 μ M
Cr****B****Figure 2**

No
tumors



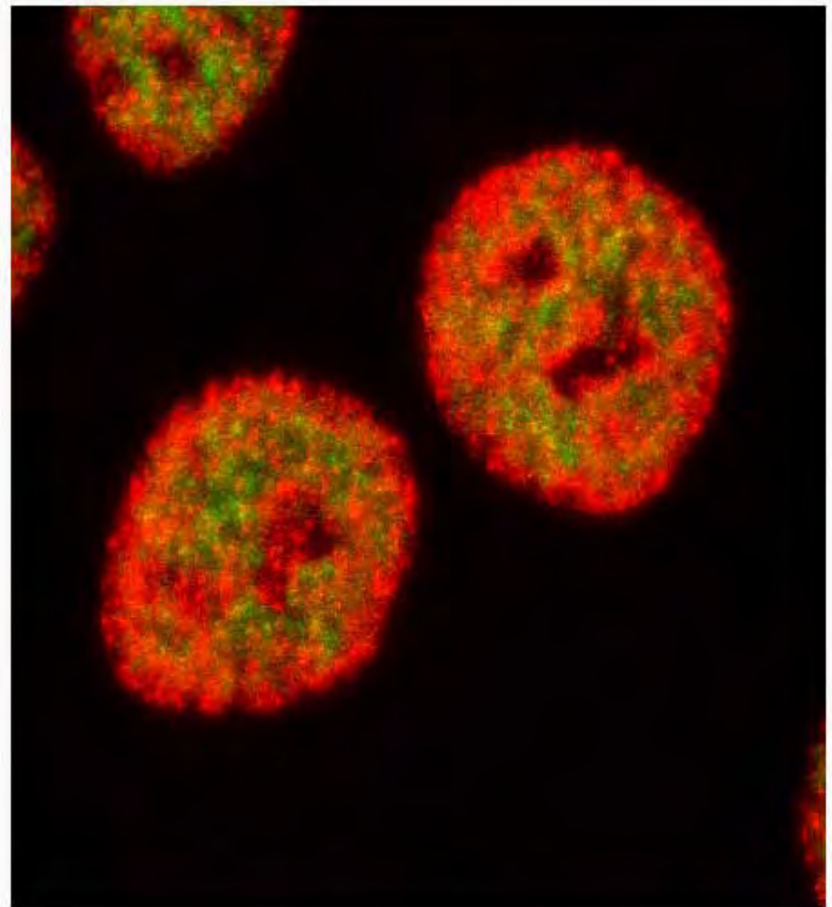
tumors
in nude
mice





Control

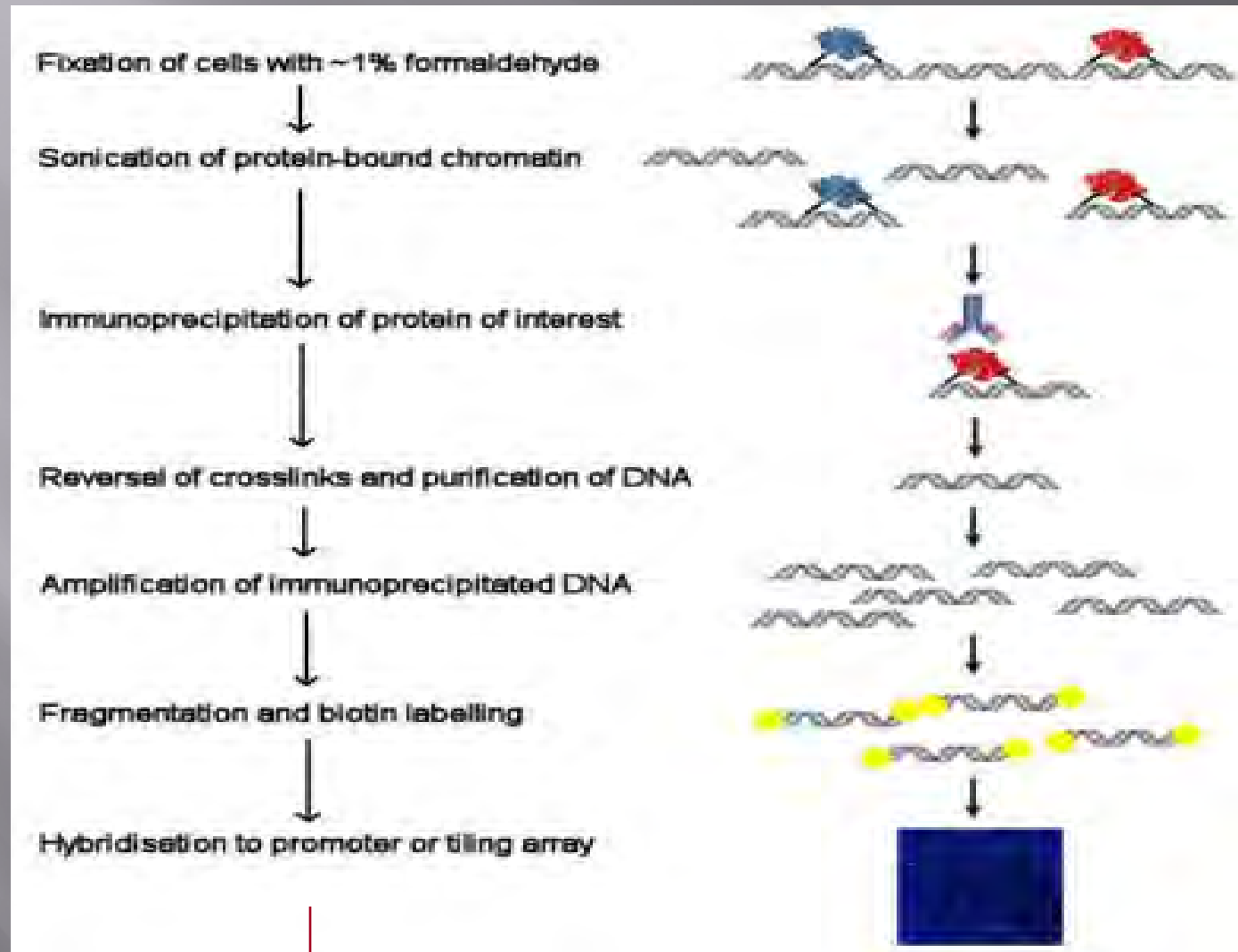
Red=H3K9me2



1 mM Ni

Green=H3K4me3

Chip-on-chip and Chip-Seq



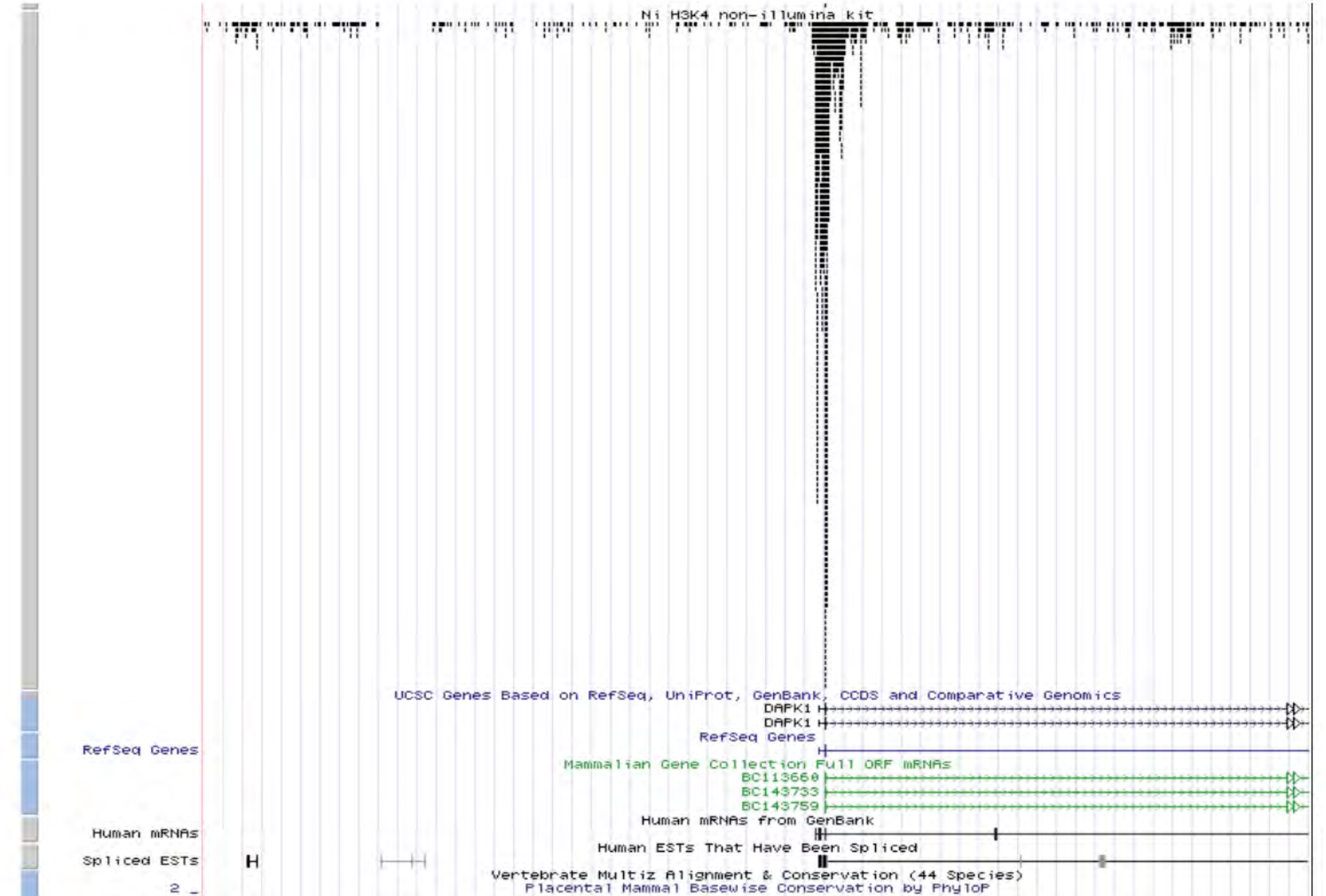
Chip-Seq sequence 36 BP DNA

Correlation of increases of H3K9me2 in gene promoter and Gene expression

strand	annotation	Symbol	Chromosome	Start	End	expression
-	IL12B	NM_002187	5	158694368	158694540	↓ 8.4
-	C10orf86	NM_01761	10	123730785	123730944	↓ 3.3
+	CD3EAP	NM_01209	5	96056126	96056327	↓ 3.5
+	CCT7	NM_01590	7	100312376	100312519	↓ 3.0

Correlation of increases of H3K4me3 in gene promoter and Gene expression

strand	annotation	Symbol	Chromosome	Start	End	Gene expres sion
+	NDRG1	NM_006096	8	134,376,200	134,377,500	↑ 21.2
+	CA9	NM_001216	9	35663914	35671151	↑ 6.5
+	STC2	NM_003714	5	172,685,450	172,685,600	↑ 3.5
+	EGLN3	NM_022073	14	33463173	33490036	↑ 3.3

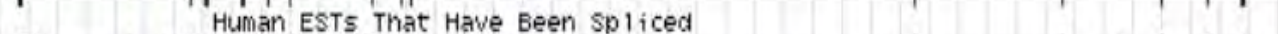
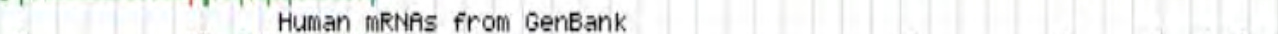
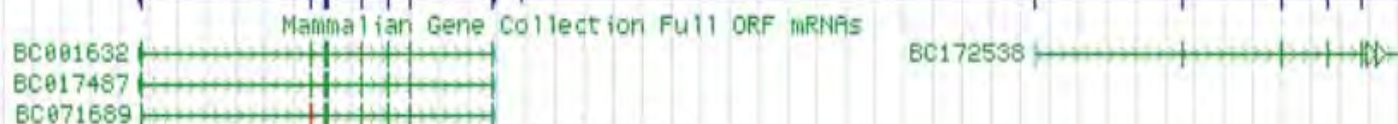


RefSeq Genes

Human mRNAs

Spliced ESTs

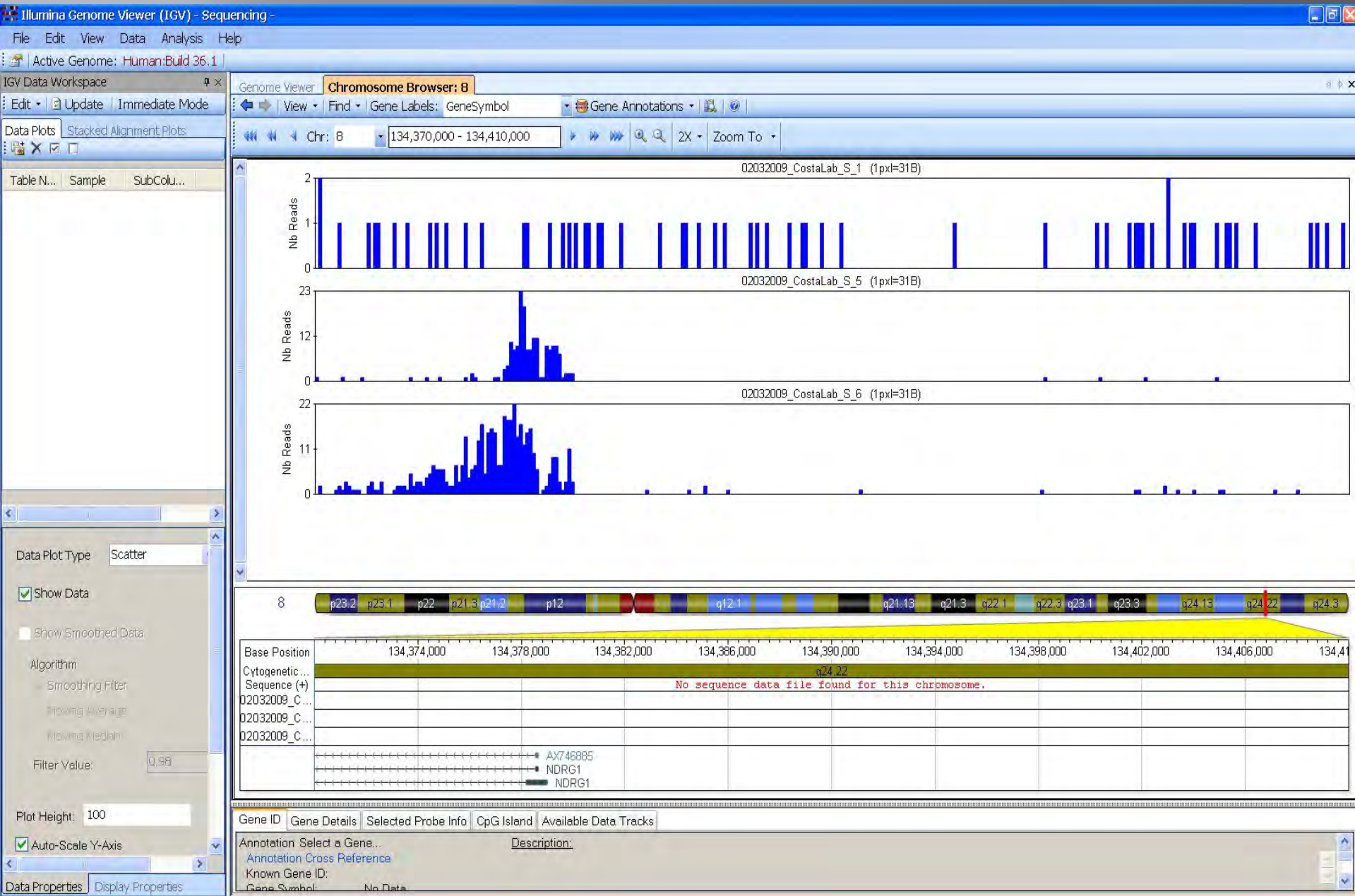
UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics



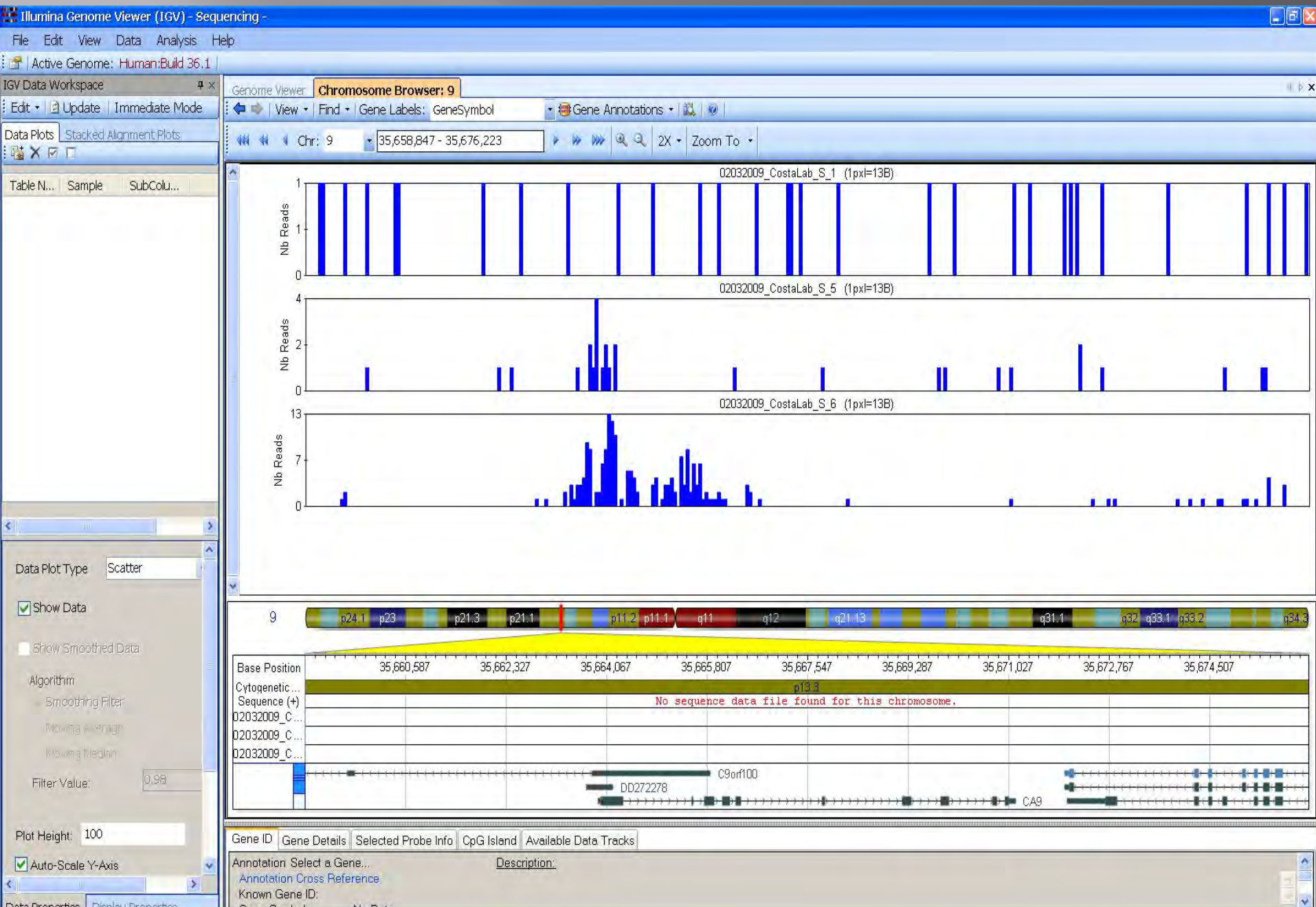
Vertebrate Multiz Alignment & Conservation (44 Species)

Placental Mammal Basewise Conservation by PhyloP

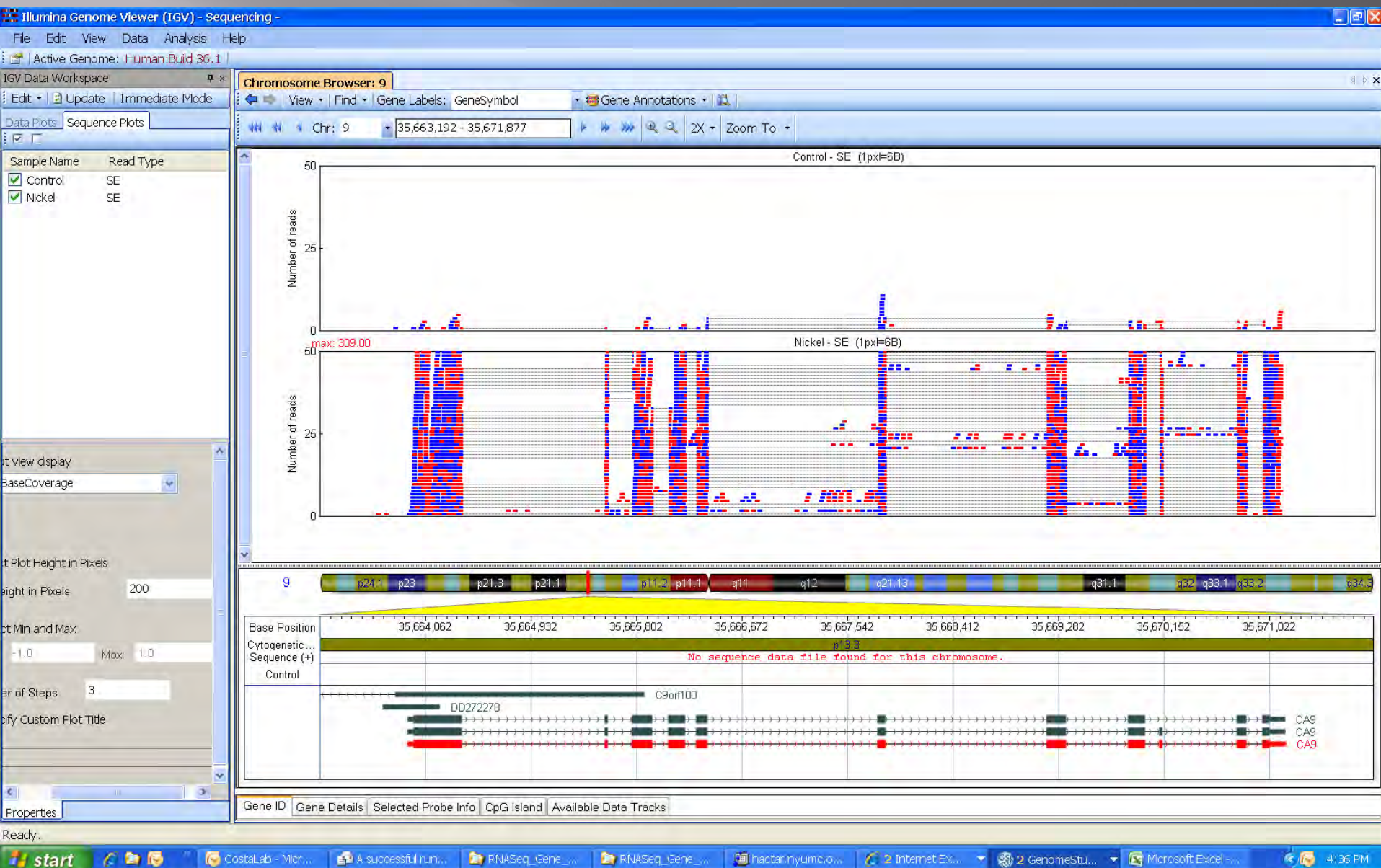
Chip-Seq H3K4 trime NDRG1/Cap43



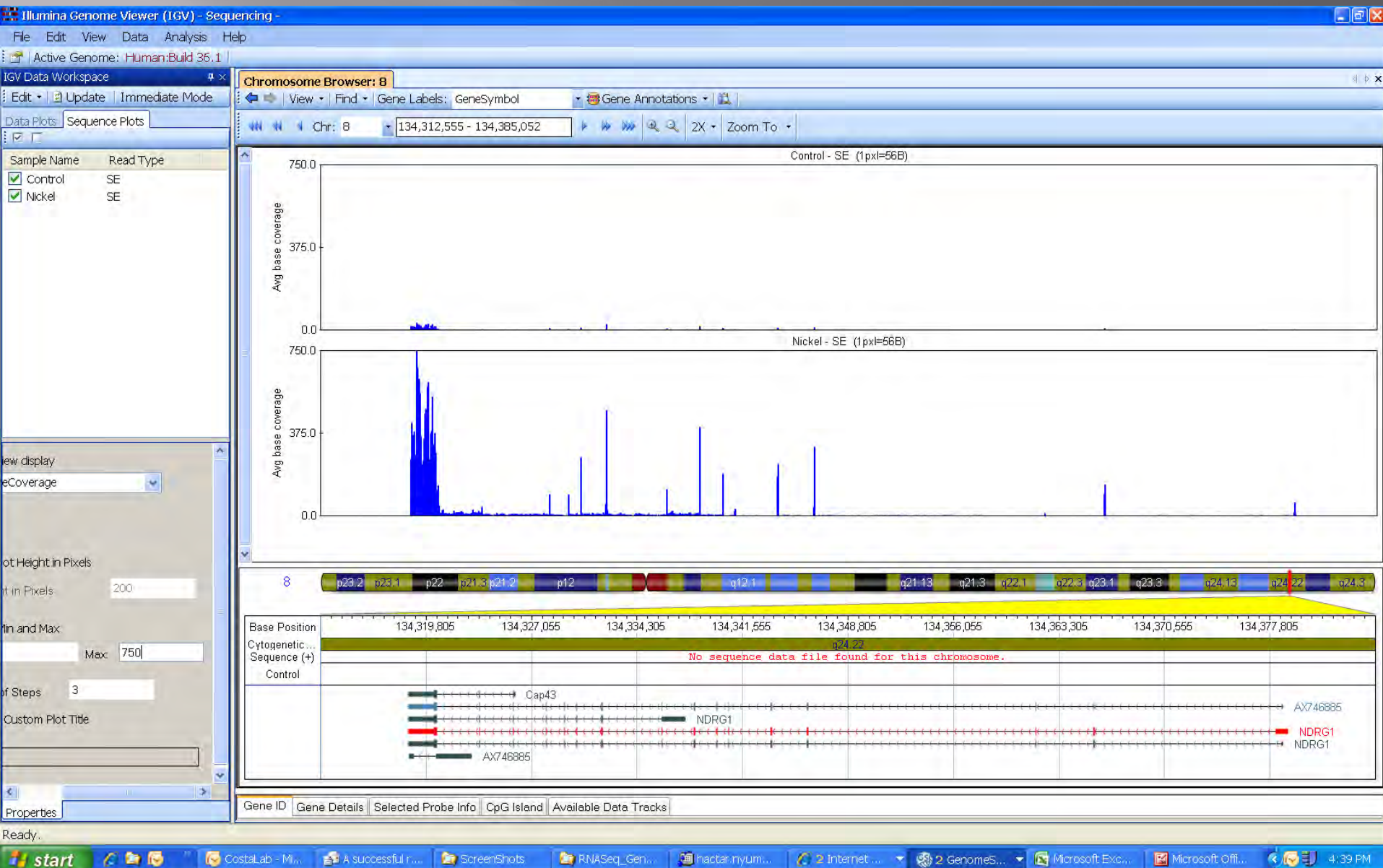
Chip-Seq H3K4 trimeth CA-9



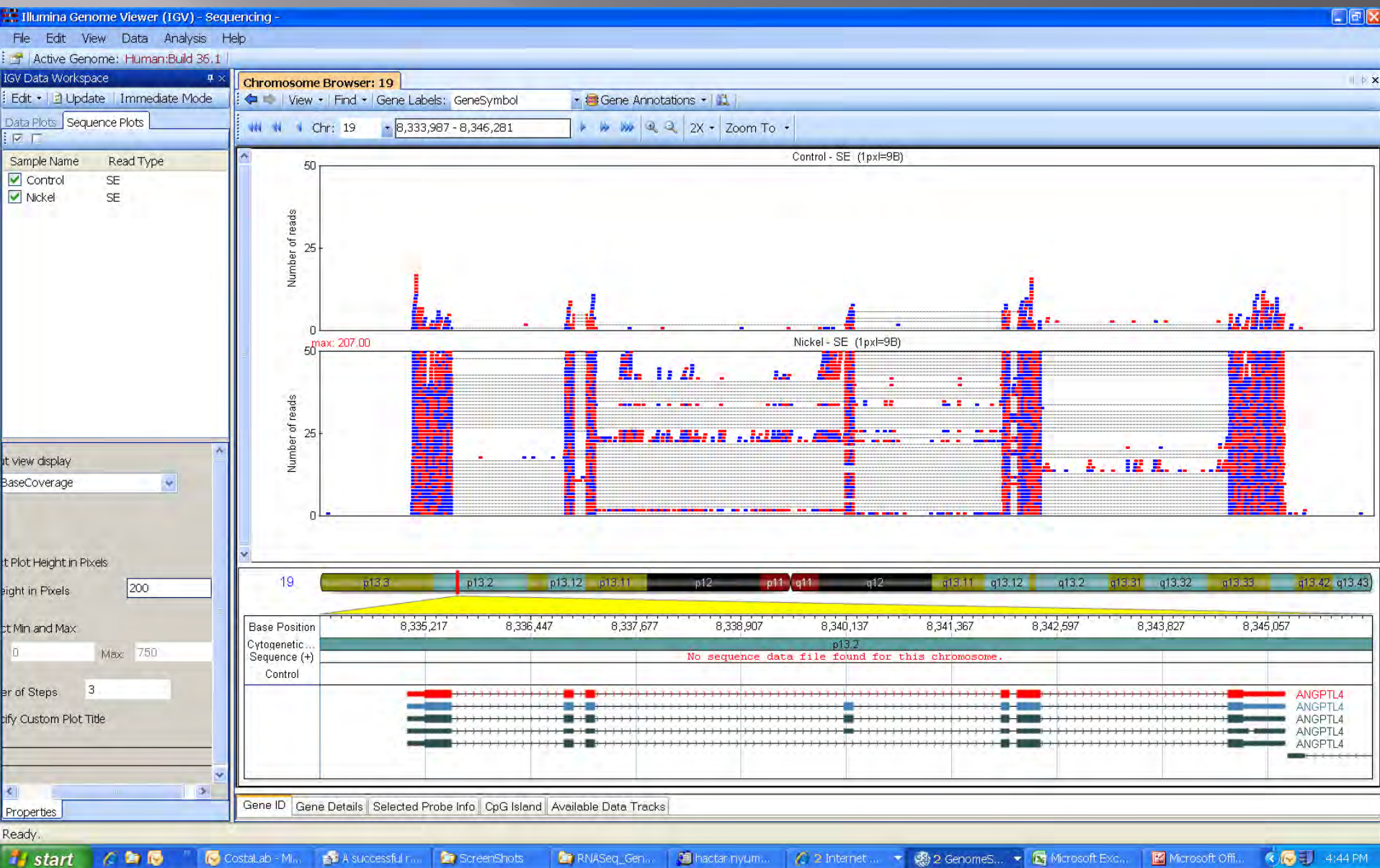
RNA-Seq +/- Nickel A549 Cells CA-9



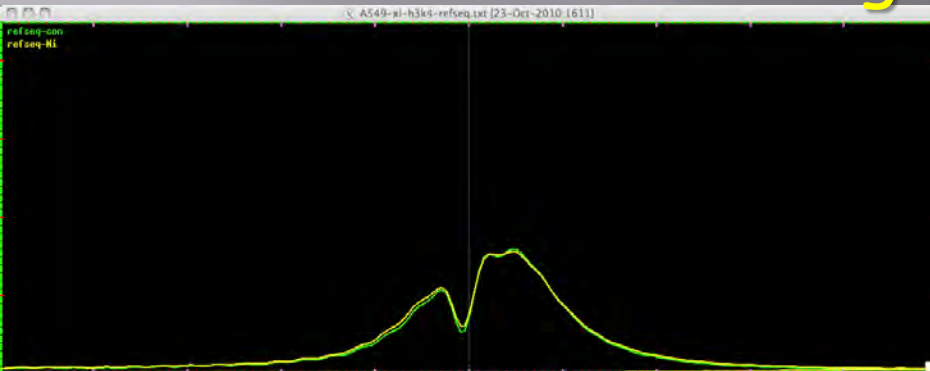
RNA-Seq +/- Nickel A549 Cells NDRG1/Cap43



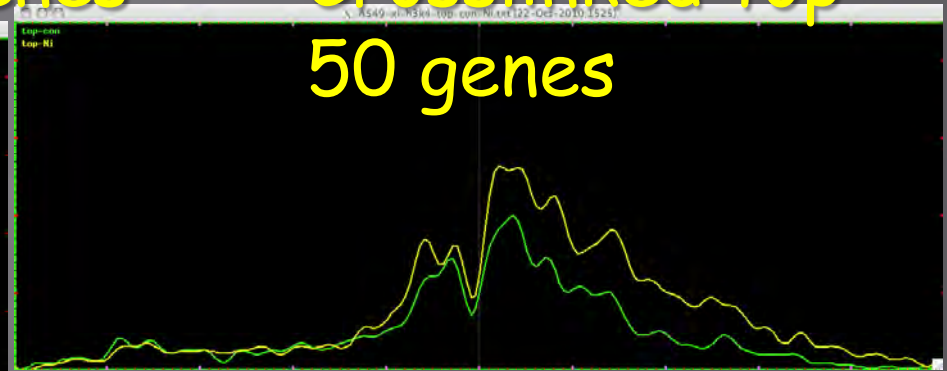
RNA-Seq +/- Nickel A549 Cells ANGPTL4



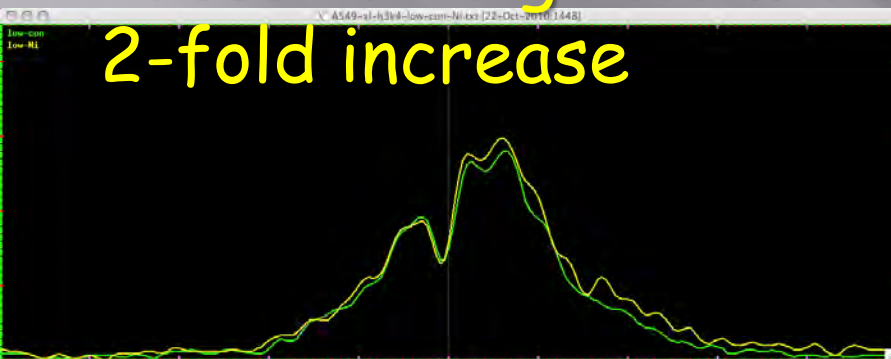
Crosslinked all genes



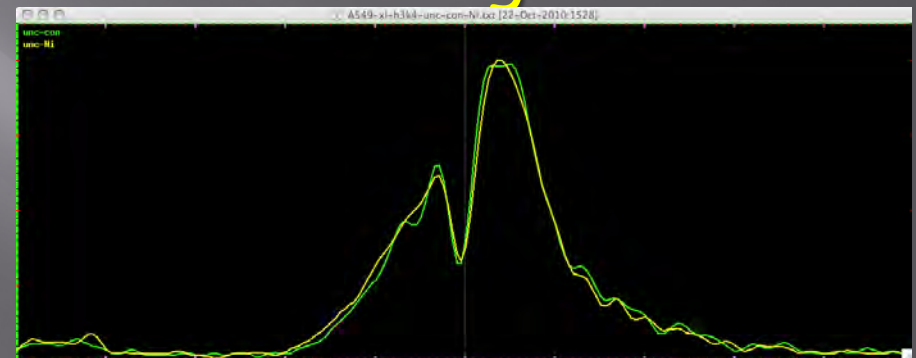
Crosslinked top 50 genes



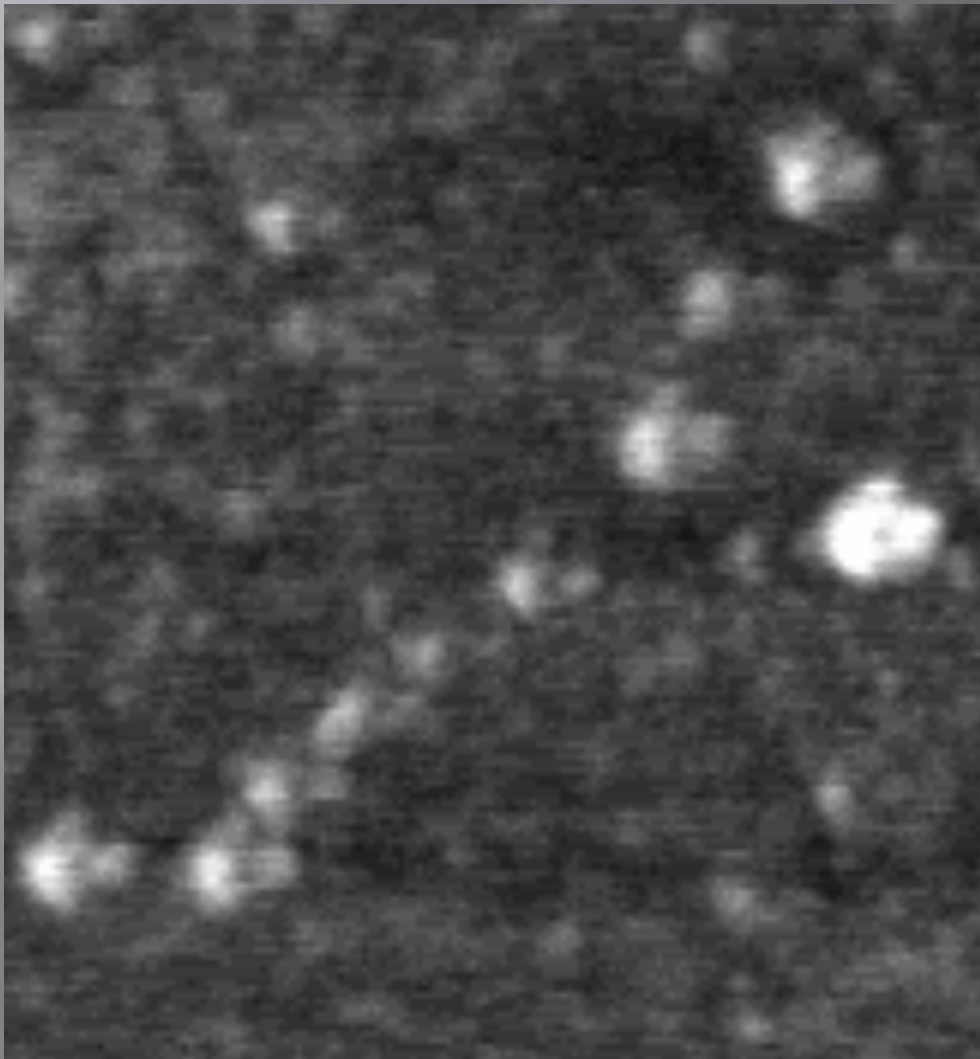
Crosslinked genes with 2-fold increase



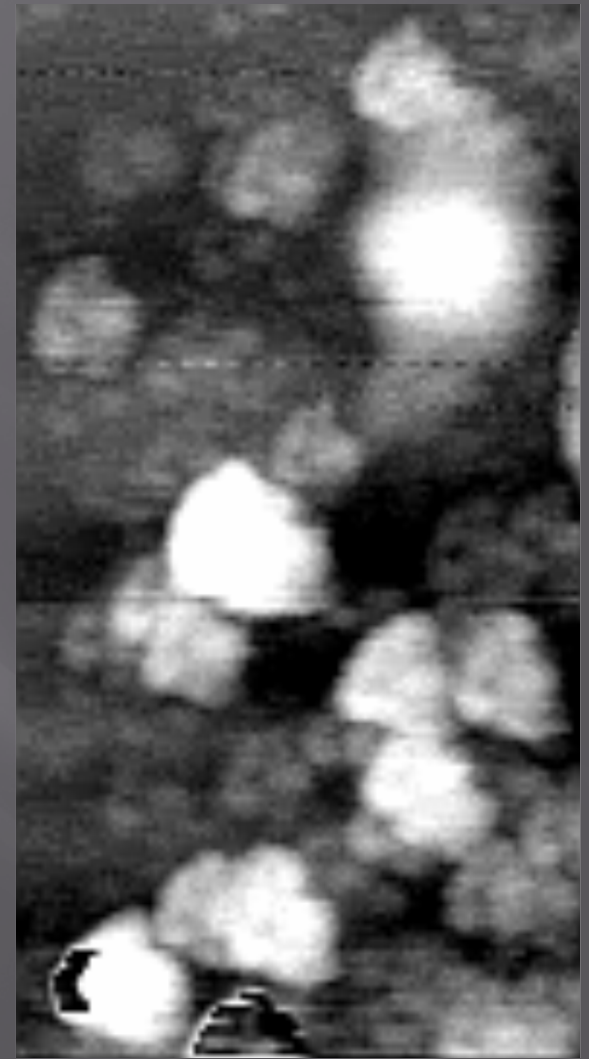
Crosslinked genes no

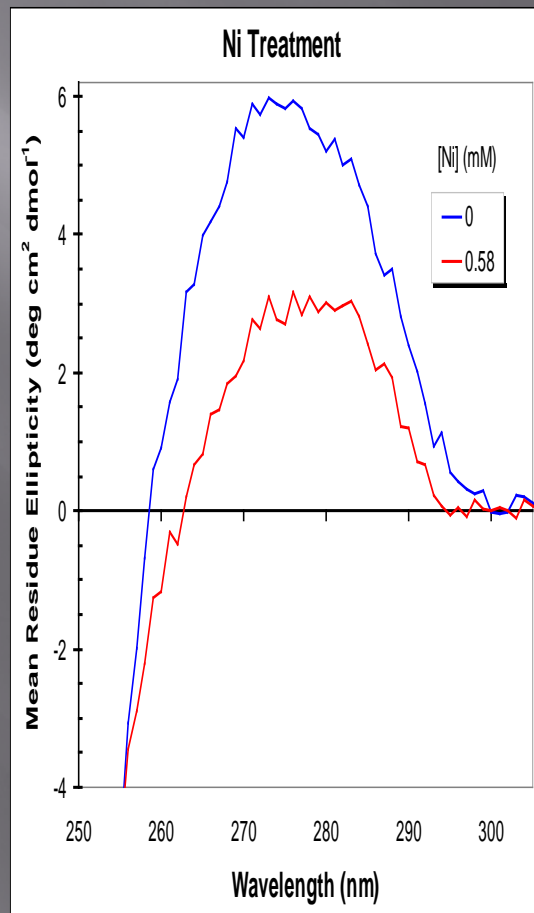
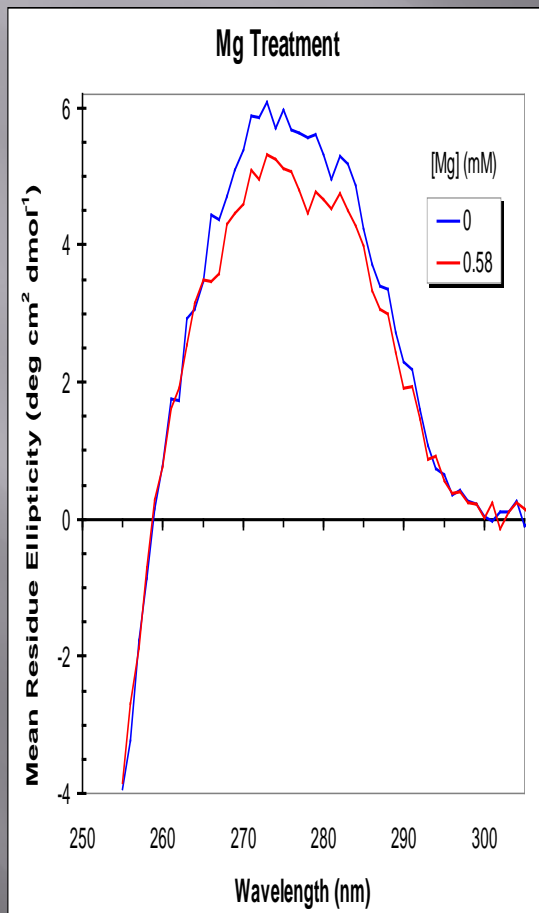


No Cation

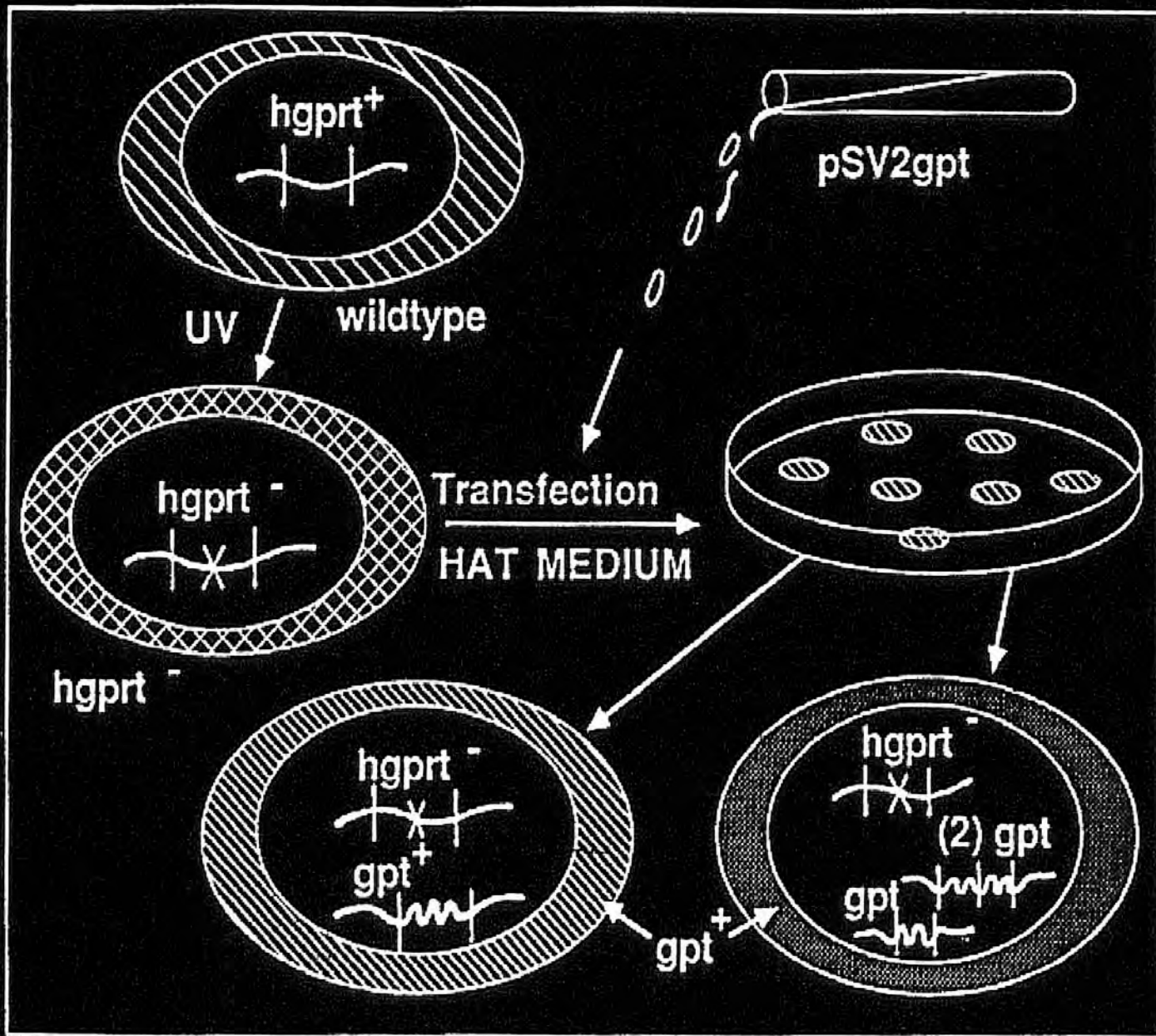


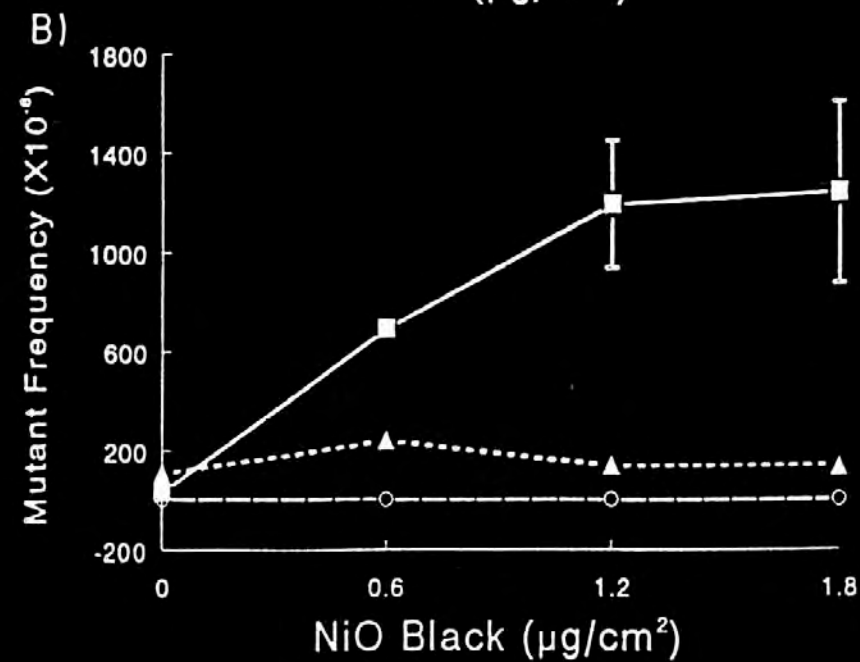
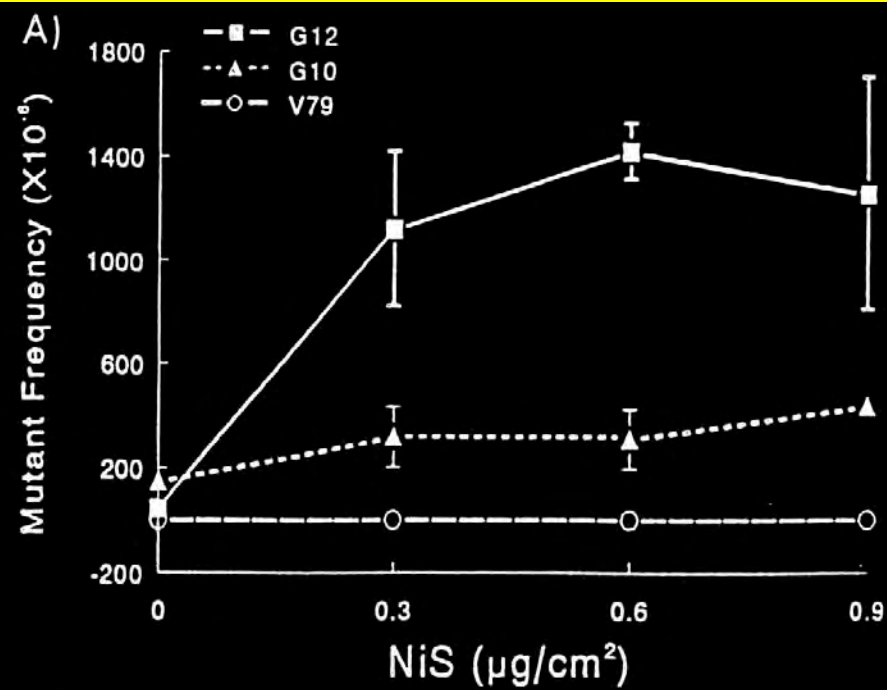
1.1mM Mg^{+2}

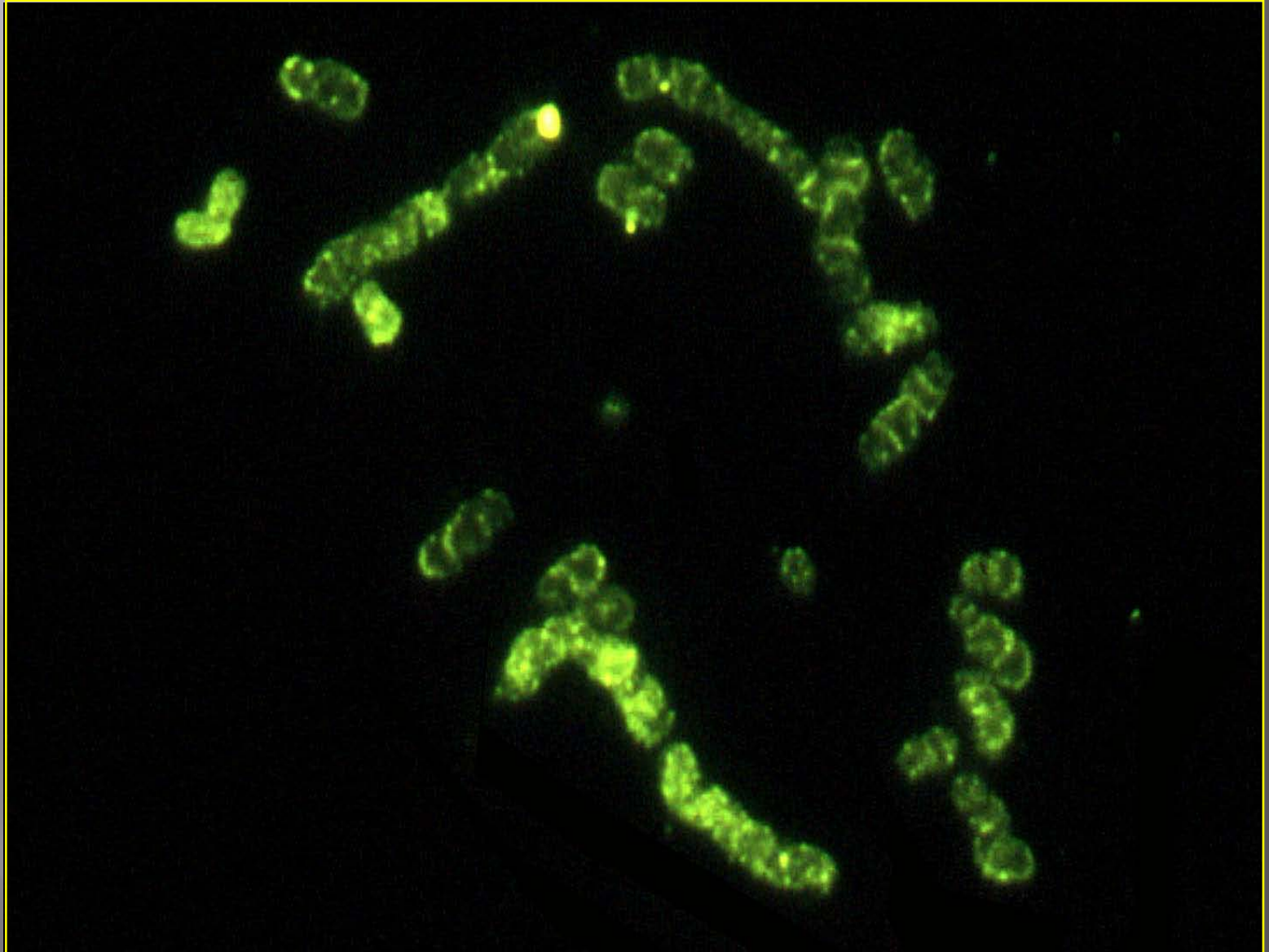




CD spectral difference of dodecanucleosome (12-mer) samples in 0 or 0.58 mM divalent cation. The left panel shows Mg²⁺-treated and the right panel shows Ni²⁺-treated oligonucleosomes. In each case the top curve is the untreated oligonucleosomes and the lower curve is divalentcation-treated oligonucleosomes.







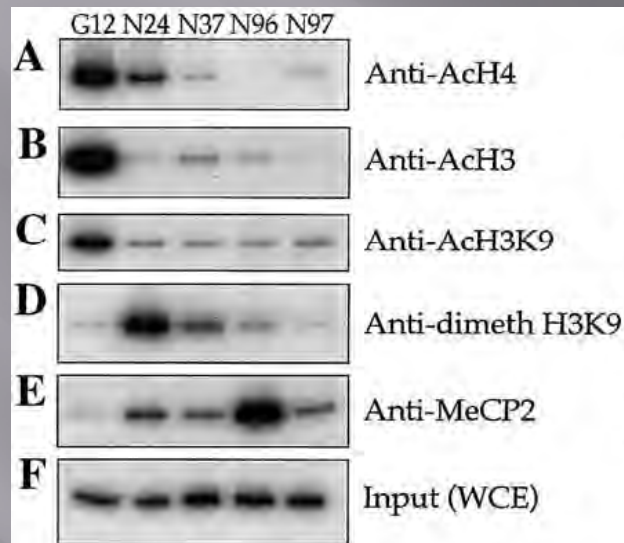
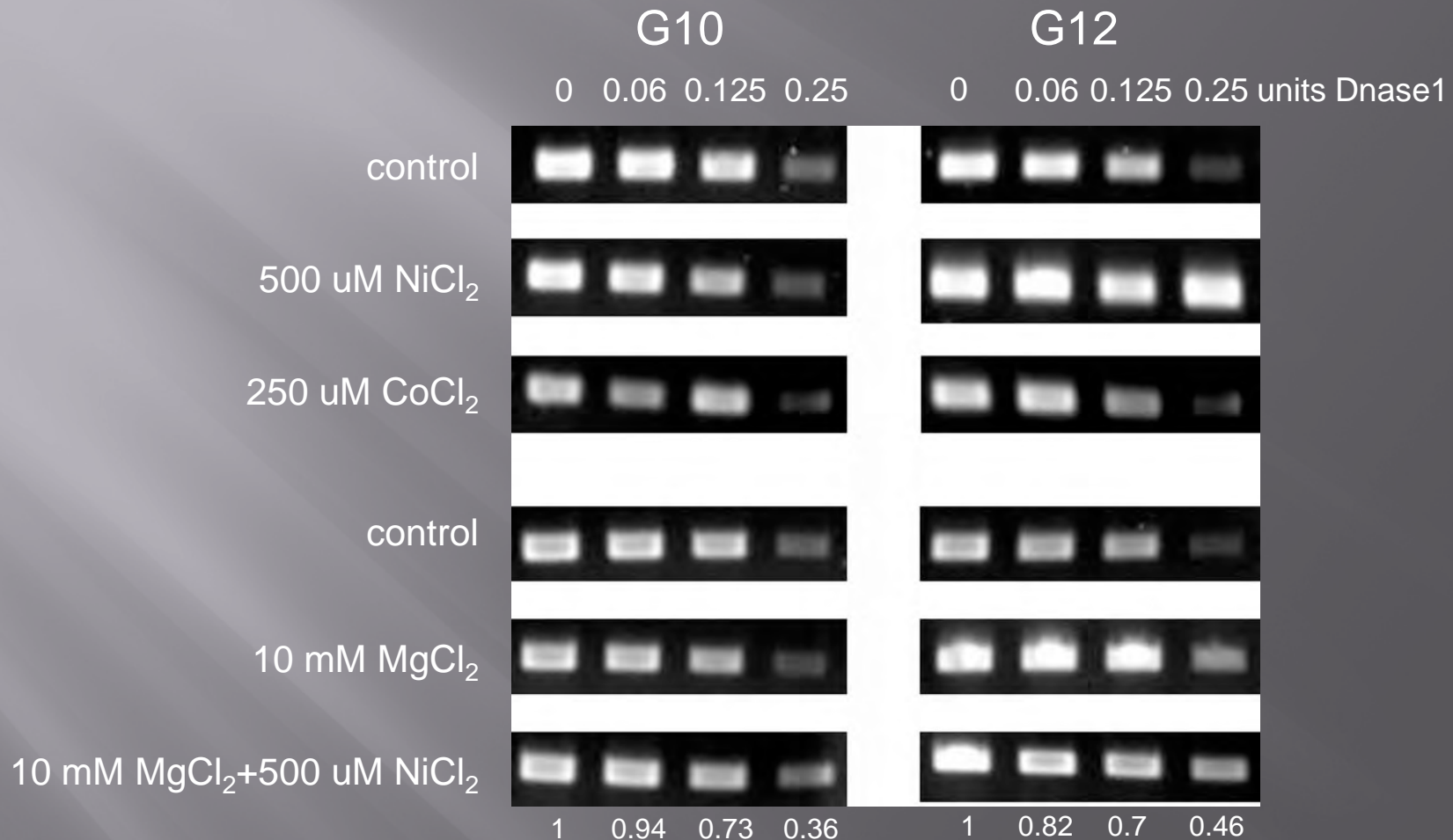


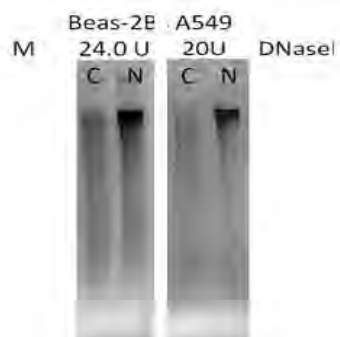
Figure 4. Analysis of chromatin proteins associated with Ni-silenced *gpt* gene using the chromosome immunoprecipitation assay. The ChIP assay was performed with anti-acetylated H4 (A), anti-acetylated H3 (B), anti-acetylated H3K9 (C), anti-dimethyl-H3K9 (D), and anti-MeCP2 (E). Input DNA fractions were amplified by PCR to adjust for chromatin loading (F). A representative gel is shown but similar results were observed in three replicate experiments. G12 indicates the wild-type clone with *gpt* expression while the N24, N37, N96, and N97 are nickel induced *gpt*-silenced clones.

GPT Dnase1 Protection Assay

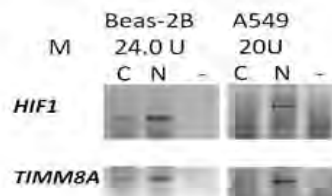


* 0.125 units Dnase1

** 0.06 units Dnase1



C- control
N-0.25mM NiCl₂ 75 min on ice



•DNaseI 20min 37C buffer with 200uM CaCl₂

Figure 10. Effect of nickel chloride on the condensation of chromatin in BEAS-2B Cells and A549 cells.

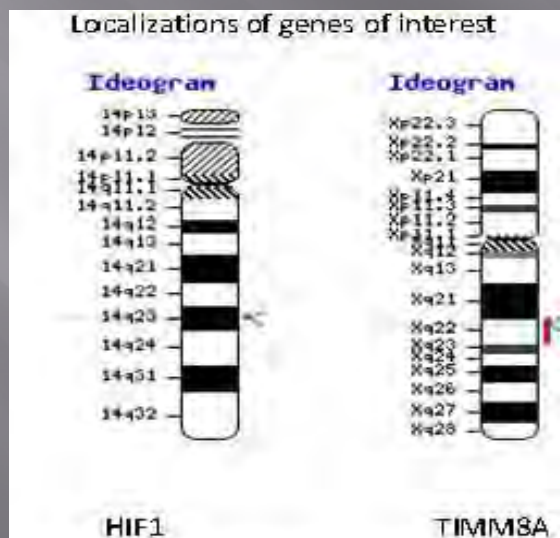
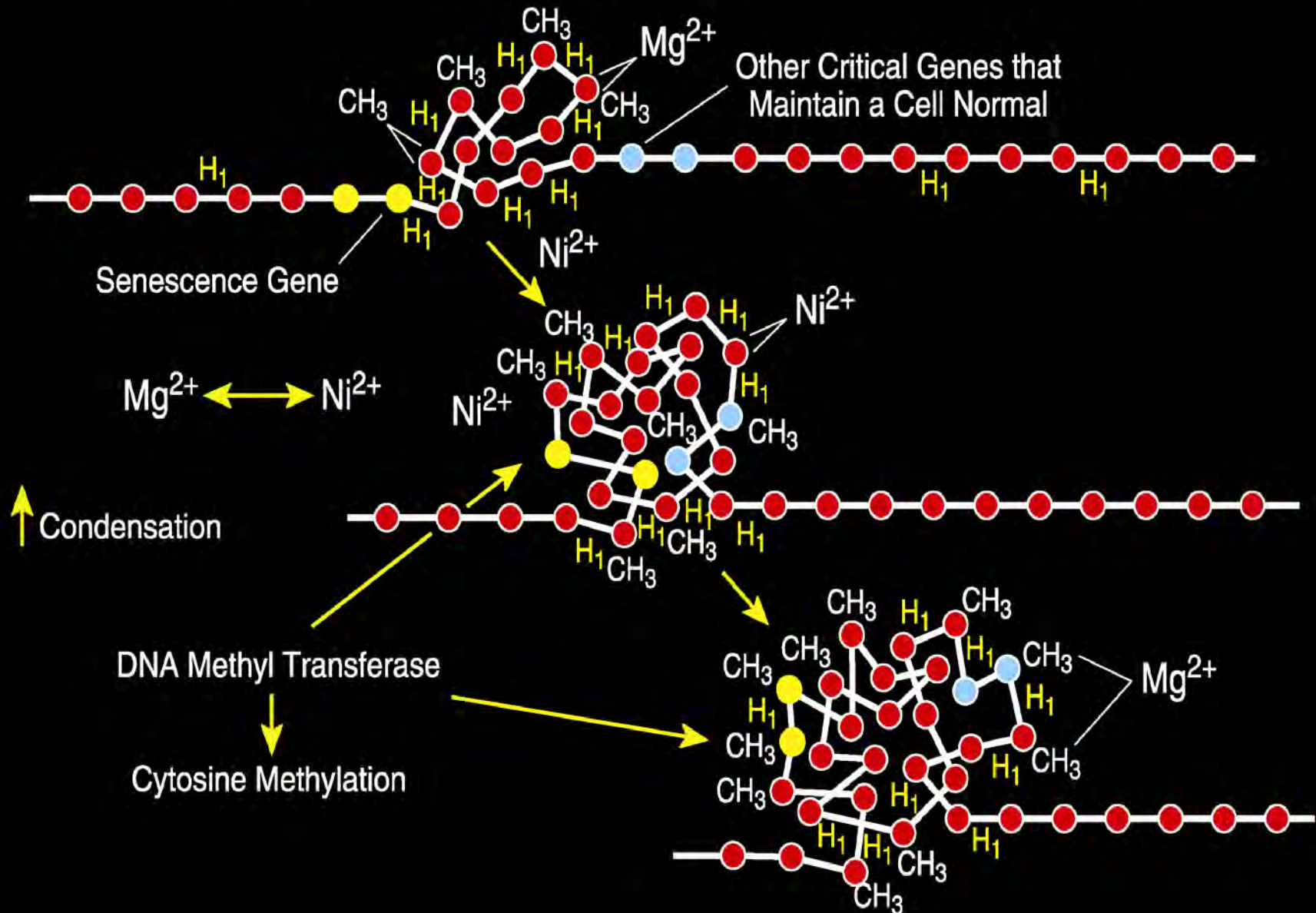


Figure 11. Chromosomal location of the HIF-1 alpha and TiMM8A gene.

Model for Transcriptional Inactivation by Ni^{2+}



Carcinogenesis vol.29 no.9 pp.1831–1836, 2008

doi:10.1093/carcin/bgn063

Advance Access publication March 4, 2008

Arsenite alters global histone H3 methylation

**Xue Zhou[†], Hong Sun[†], Thomas P. Ellen, Haobin Chen and
Max Costa***

Modulation of histone methylation and *MLH1* gene silencing by
hexavalent chromium

Toxicology and Applied Pharmacology 237 (2009) 258–266

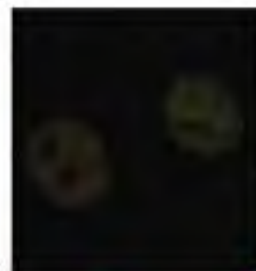
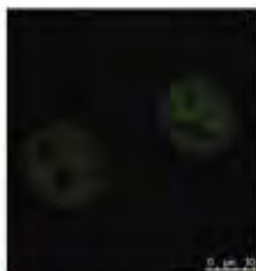
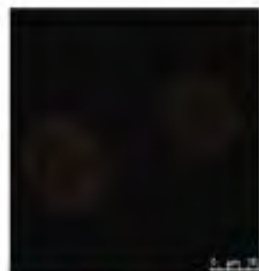
Hong Sun¹, Xue Zhou¹, Haobin Chen, Qin Li, Max Costa*

Effects of nickel, chromate, and arsenite on histone 3 lysine methylation

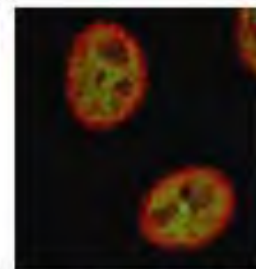
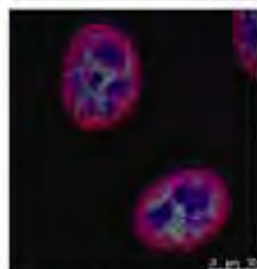
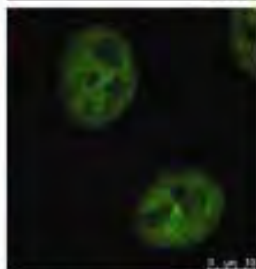
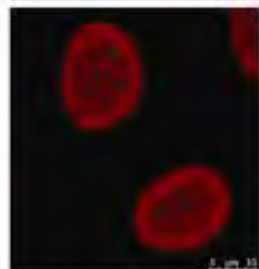
Xue Zhou, Qin Li, Adriana Arita, Hong Sun, Max Costa*

Toxicology and Applied Pharmacology 236 (2009) 78–84

Control



5 μ M Cr



H3K9me2

H3K4me3

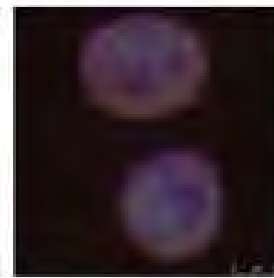
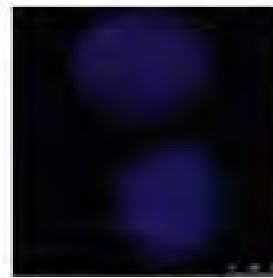
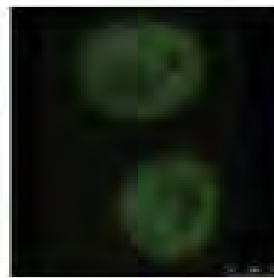
DAPI

Merge

H3K9me2+H3K4me3

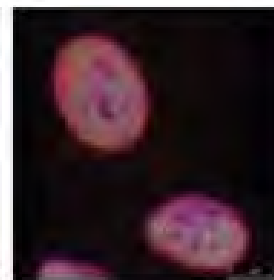
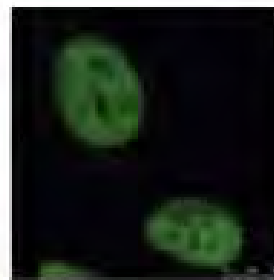
C

Control



H3K4me3

5 μ M As



H3K9me2

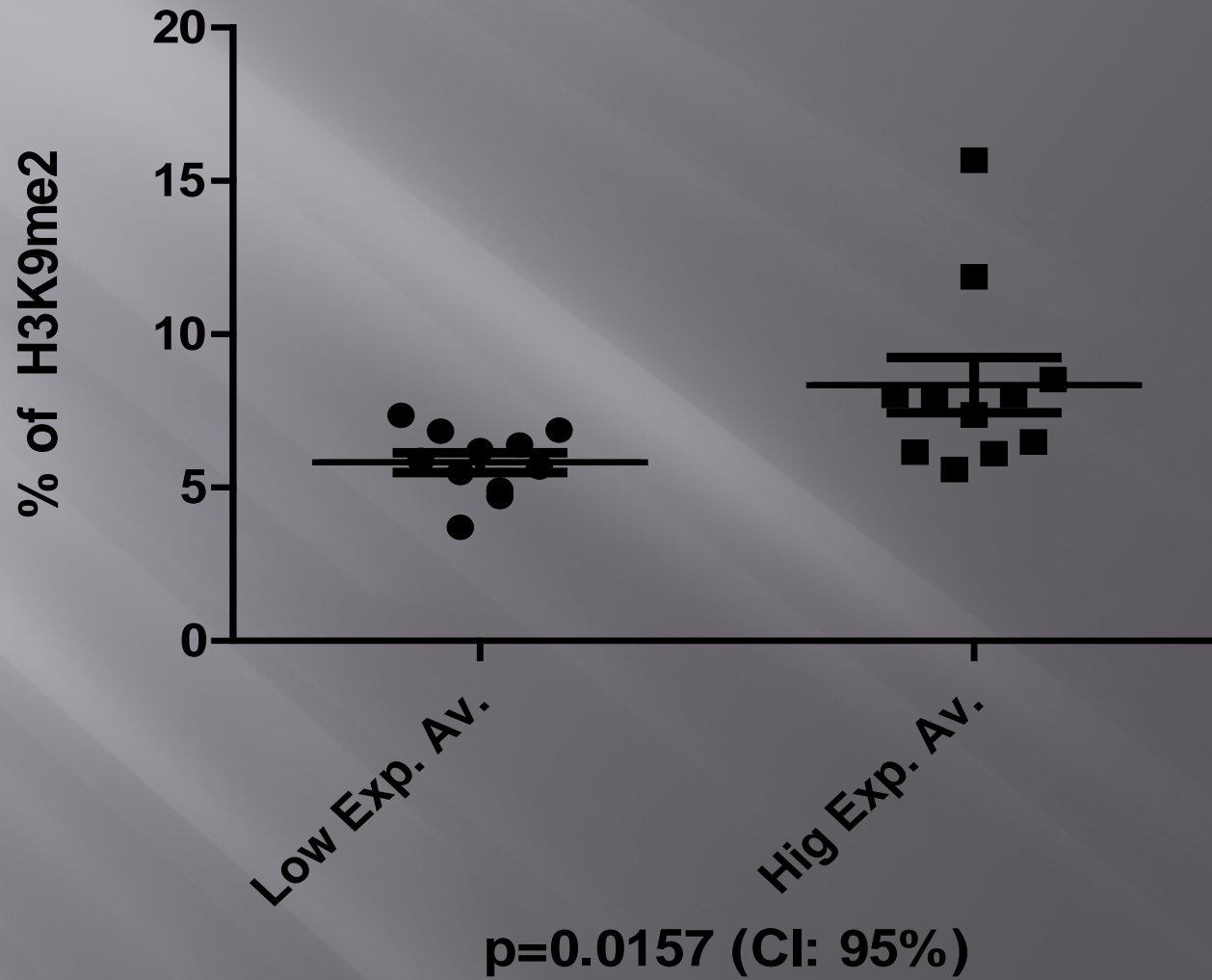
H3K4me3

DAPI

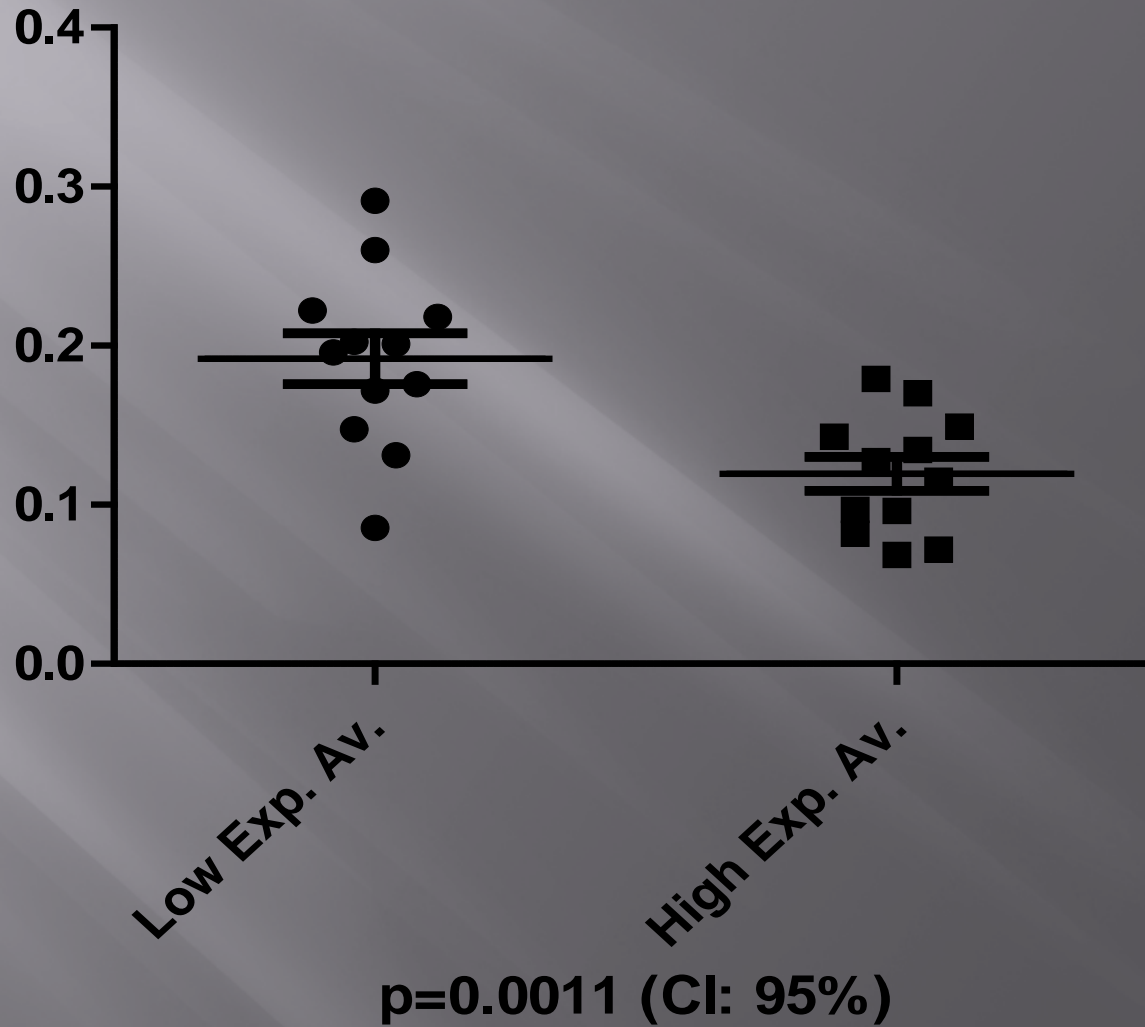
Merge

H3K9me2 +
H3K4me3

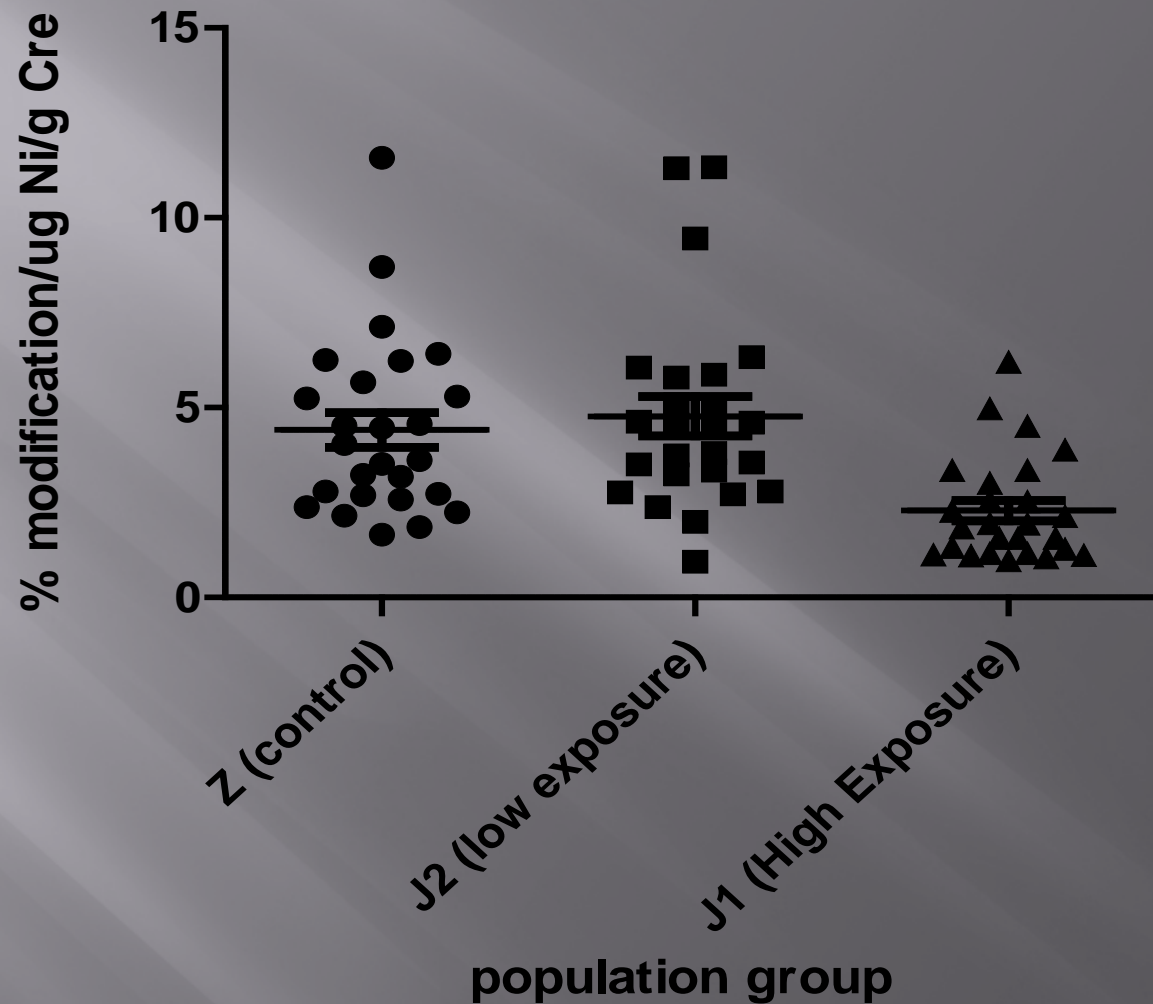
H3K9me2 All runs Av. with Std.



H3K9acetyl Av. of all runs



H3K9me2



$p^* = 0.6056$

$p^{**} = 0.0002$

$p^* = t \text{ test (Z vs J2)}$ $p^{**} = t \text{ test (Z vs J1)}$

SUMMARY

- Nickel ions inhibit the dioxygenase histone demethylases leading to increased H3K4 tri and H3K9 di which increases or decreases the expression of specific genes, respectively
- Nickel ions bind and displace the Fe in the His-His Glu facial triad at the active site of dioxygenases such as ABH2
- Mapped genomic positions of H3K4 tri and H3K9 di changes induced by Ni using Chip-on-chip and Chip-Seq technology (correlates with gene expression changes)
- SPRY2 which inhibits ERK signaling is a direct target of histone demethylase JMJD2A
- SPRY2 is epigenetically suppressed in Nickel induced transformed BEAS2B cells (By chronic inhibition of JMJD2A?) and overexpression of SPRY2 reversed the transformed phenotype
- Gene expression changes are very metal specific in Normal Human Bronchial Epithelial cells transformed by Nickel and Chromate
- Nickel induces condensation of chromatin and silencing of genes near or in heterochromatin

Acknowledgements

▣ Haobin Chen

Kathrin Kiok

▣ Thomas Kluz

Yana Chervona

▣ Thomas Ellen

▣ Hong Sun

▣ Adriana Arita

▣ Mike Marone

▣ Nitai Giri



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